EARLY DIAGNOSTIC MARKERS FOR NEONATAL SEPSIS: COMPARING PROCALCITONIN AND C-REACTIVE PROTEIN WITH THE GOLD STANDARD CULTURE METHOD

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

In partial fulfillment of the regulations
for the award of the degree of

M.D.(MICROBIOLOGY)
BRANCH – IV

MADRAS MEDICAL COLLEGE
THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI – TAMILNADU.

APRIL 2016

CERTIFICATE

This is to certify that this dissertation titled **"EARLY DIAGNOSTIC MARKERS FOR NEONATAL SEPSIS:COMPARING PROCALCITONIN AND C-REACTIVE PROTEIN WITH THE GOLD STANDARD CULTURE METHOD"** is a bonafide record of work done by DR. R.KESAVAN, during the period of her Post Graduate study from 2013 to 2016 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai- 600003, in partial fulfillment of the requirement of M.D MICROBIOLOGY degree Examination of The Tamilnadu Dr. M.G.R Medical University to be held in April 2016.

Dr.R.VIMALA., M.D.,
Dean
Madras Medical College &
Government General Hospital,
Chennai - 600 003.

Dr.G.MANGALA ADISESH,M.D.,
Director I/C,
Institute of Microbiology,
Madras Medical College&
Government General Hospital
Chennai – 600 003.

DECLARATION

I declare that the dissertation entitled **"EARLY DIAGNOSTIC MARKERS FOR NEONATAL SEPSIS: COMPARING PROCALCITONIN AND C-REACTIVE PROTEIN WITH THE GOLD STANDARD CULTURE METHOD"** submitted by me for the degree of M.D. is the record work carried out by me during the period of October 2014 – August 2015 under the guidance of **Prof.** Dr. S.Thasneem Banu, M.D., Professor, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in April 2016.

Place : Chennai
Date:

Signature of the candidate

(Dr.R.KESAVAN)

Signature of the guide
Prof.Dr.S.THASNEEM BANU, M.D.,
Professor
Institute of Microbiology
Madras Medical College, Chennai-3

ACKNOWLEDGEMENT

I humbly submit this work to the Almighty who has given the health and ability to pass through all the difficulties in the compilation and proclamation of this blue print.

I wish to express my sincere thanks to our Dean, **Dr.R.Vimala M.D.,** for permitting me to use the resources of this institution for my study.

I owe special thanks to **Prof.Dr.Mangala Adisesh, M.D.,** Director I/C and Professor, Institute of Microbiology for her support, invaluable suggestions, erudite guidance in my study and for being a source of inspiration in my endeavours.

I feel fortunate to work under the guidance of **Prof. Dr.S.Thasneem banu, M.D.,** for her valuable suggestions and great support throughout my study.

I would like to thank my Professors **Dr.S.Vasanthi M.D., Dr.U.Umadevi M.D., Dr. R.Vanaja M.D.,** for their valuable assistance in my study.

I express my gratitude to our former Director, **Prof.Dr.G.Jayalakshmi, M.D.,DTCD.** and former Professors

Dr.T.Sheila Doris M.D., and **Dr.K.Muthulakshmi M.D.**,for their guidance and support.

I extend my whole hearted gratitude and special thanks to my Assistant Professor **Dr.K.Usha Krishnan M.D.,** for her valuable guidance and constant support in my study.

I also express my thanks to our Assistant professors **Dr.R.Deepa M.D., Dr.N.Rathnapriya M.D., Dr.C.S.Sripriya M.D., Dr. David Agatha M.D.**,**Dr.N.Lakshmipriya M.D.,DCH., Dr.K.G.Venkatesh M.D,** and **Dr.B.Natesan M.D.,DLO.,** for their immense support in my study.

I extend my thanks to Professor **Dr.N.Devasena M.D.,** Department of Microbiology, Institute of Child Health(ICH) ,Egmore.

My sincere thanks to **Dr. J .Kumudha,M.D (Pediatrics)., HOD.**, &Professor, Department of Neonatology for permitting me to carry out my study.

I hereby express my gratitude to all the technical staff for their help throughout my study.

I would like to thank my department colleagues and friends for their constant support and co-operation.

I would like to thank the Institutional Ethics Committee for approving my study.

Finally I am indebted to my parents **Mr.N.Radhakrishnan, Mrs.V.A.Deivayanai,** my wife **Mrs.A.Shobana M.Sc.,B.Ed,** my sister in law **Miss.A.Divya,** my children **K.Jeevanesh** and **K.Nithish** who have sacrificed their family time and let me concentrate on my study. I thank them for being solid pillars of everlasting support and encouragement to me.

TABLE OF CONTENTS

ABSTRACT

Introduction: Isolation of the causative microorganisms by using blood culture has been the golden standard method for its diagnosis, the result is ready only after 72 hrs after the sampling and during this period, it is necessary to treat the suspicious infants for sepsis with antibiotics on the basis of the clinical symptoms and the risk factors. Early identification of bacterial infections by Quantitative procalcitonin estimation in neonatal sepsis cases and appropriate antibiotic therapy will reduce the morbidity and mortality.

Aims and objectives: The aim of the study is to determine the diagnostic performance of procalcitonin and C-reactive protein for the early diagnosis of Neonatal Septicemia.

Materials and methods: This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College, Rajiv Gandhi Government General Hospital (RGGGH), Chennai in association with Neonatal unit Institute of Child Health, Egmore from October 2014 to August 2015.Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were included in this study. Blood collected from each neonate for sepsis work up. Blood culture and antibiotic sensitivity pattern were carried out. In this study, the estimation of serum C - reactive protein was done by GenX CRP

Turbilatex Quantitative method. The measurement of serum procalcitonin was done by QDx Instacheck PCT

Results: In this study, the estimation of serum C-Reactive Protein was done by GenX CRP Turbilatex Quantitative method. The sensitivity of CRP in detecting sepsis was 62.5% , its specificity was 65.3%, its positive predictive value was 35.7% and its negative predictive value was 84.4%. The correlation between serum PCT and CRP was compared .Higher proportion of the neonates with sepsis had raised PCT than those without sepsis. The sensitivity of serum procalcitonin (87.5%) for detection of neonatal sepsis was higher than the sensitivity of CRP (62.5%). In most of the culture positive cases, the other sepsis screening tests were negative , but the level of PCT was elevated.

Conclusion: Infants who are suspected to have neonatal sepsis may lead to unnecessary admission and increased antibiotic consumption, a higher incidence of the side effects due to their use, increased resistance to the antibiotics, a long hospitalization, the separation of the infants from their mothers and increased health costs. The findings of the present study confirm that serum levels of PCT was a more reliable marker than the serum levels of CRP and WBC counts in the early diagnosis of neonatal sepsis and in the evaluation of the response of the disease to the antibiotic therapy.

INTRODUCTION

Invasion of the bloodstream by microorganisms constitutes one of the most serious situations in infectious disease. Microorganisms present in the circulating blood –whether continuously, intermittently or transiently are a threat to every organ in the body [1].

Septicemia indicates bacteria are present in the blood, producing an infection and reproducing within the blood stream that can have serious immediate consequences including shock, multi organ failure, Disseminated intravascular coagulation (DIC) and death [1] .

Sepsis is the commonest cause of neonatal mortality, it is responsible for about 30 – 50% of the total neonatal deaths in developing countries [2] [23]. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes [3]. Sepsis related mortality is largely preventable with rational antimicrobial therapy and aggressive supportive care

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first 30 days of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections. [4]

Superficial infections like conjunctivitis and oral thrush are not usually included under neonatal sepsis.

The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live birth. The NNPD network comprising of 18 tertiary care neonatal units across India found sepsis to be one of the commonest causes of neonatal mortality contributing to 19% of all neonatal death [7].

Microbial colonization of newborn infants begins immediately after birth. Infants skin is colonized by flora derived from the body of the mother and other depending on the first suitable organism to arrive at a particular site as well as factors such as the type of delivery, amount of vernix caseosa present at birth, the type of nourishment received (breast milk or formula) and the degree of exposure in the hospital environment [8].

Neonatal sepsis can be divided into two main subtypes depending on whether the onset is during the first 72 hours of life (Early onset sepsis), and after 72 hours of birth (Late onset sepsis) [9] [28].

Early onset septicemia is caused by organisms prevalent in the genital tract or in the labour room and maternity operation theatre. In the west early onset infections are mostly caused by group B *streptococci and Escherichia coli* , while in our setup most cases are due to gram negative organisms especially *E.coli, Klebsiella*, and

Enterobacter species. Majority of neonates with Early onset sepsis manifest as respiratory distress due to intrauterine pneumonia[10].

Late onset sepsis is acquired as nosocomical infection from the nursery or lying- in ward. In most cases symptoms appear by the end of first week or during second week of life. About two third cases of late onset septicaemia are caused by Gram negative organisms like *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* while the rest are Gram positive organisms including *Staphylococcus aureus* and *Coagulase Negative Staphylococci(CONS).*

Among intramural births (a baby born within premises of the centre), *Klebsiella pneumoniae* was the most frequently isolated pathogen (32.5%) followed by *Staphylococcus aureus* (13.6%). Among extramural neonates (referred from community/other hospitals) *Klebsiella pneumoniae* was again the commonest organism (27%) followed by *Staphylococcus aureus* (15%) and *Pseudomonas* (13%)[8].

Isolation of the causative microorganisms by using blood culture has been the golden standard method for its diagnosis, the result is ready only after 72 hrs after the sampling and during this period, it is necessary to treat the suspicious infants for sepsis with antibiotics on the basis of the clinical symptoms and the risk factors.

Infants who are suspected to have neonatal sepsis may lead to unnecessary admission and increased antibiotic consumption, a higher

incidence of the side effects due to their use, increased resistance to the antibiotics, a long hospitalization, the separation of the infants from their mothers and increased health costs.

An inflammatory marker such as C Reactive Protein (CRP) does not reliably differentiate between the systemic inflammatory response and sepsis. Procalcitonin (PCT), a precursor of calcitonin is a 116 amino acid protein secreted by the C cells of thyroid gland in normal situation but its levels may increase during septicemia, meningitis, pneumonia and urinary tract infection[11].

Early identification of bacterial infections by Quantitative procalcitonin estimation in neonatal sepsis cases and appropriate antibiotic therapy will reduce the morbidity and mortality.

This study was done to determine the various bacterial agents involved in neonatal sepsis cases and to identify the risk factors and to determine the antimicrobial susceptibility pattern. To compare the diagnostic performance of C- Reactive protein and procalcitonin with the gold standard culture method.

AIMS AND OBJECTIVES

- To isolate and identify the bacteria involved in Neonatal sepsis
- To study the antibacterial susceptibility pattern for the isolates.
- To know the risk factors involved in neonatal sepsis.
- To study the prevalence of neonatal sepsis cases.
- To determine the diagnostic performance of procalcitonin and C-reactive protein for the early diagnosis of Neonatal Septicemia.

REVIEW OF LITERTURE

DEFINITION

Neonatal sepsis or septicemia is the term that have been used to describe the systemic response to infection in newborn infants during the first 28 days of life [3].

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first 30 days of life [12].

EPIDEMIOLOGY OF NEONATAL SEPSIS:

The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live birth. The NNPD network comprising of 18 tertiary care neonatal units across India found sepsis to be one of the commonest causes of neonatal mortality contributing to 19% of all neonatal death [7].

The incidence of sepsis varies from 7.1 to 38[13] per 1000 live births in asia. 6.5 to 23[14]/1000 live births in Africa, 3.5 [15]to8.9[16] per 1000 live birth in south America and carribeans: 6[14] per 1000 live births in USA and Australia

COLONIZATION OF ORGANISMS CAUSING NEONATAL INFECTION:

The fetus normally encounters no organisms during development and the newborn infant become harmlessly colonized by bacteria

acquired from the birth canal and the environment. This is because of the barrier to infection provided by the placenta and membranes, the low pathogenicity of of colonizing microorganisms and the relative competence of the babies defence mechanisms. It is usually when one or other of these factors is altered that fetal or neonatal infection occurs.

Colonization of healthy infants:

The nature of colonizing organisms is determined by the pattern of flora in the birth canal and in the environment. Babies born at home are colonized by organisms, derived primarily from the mother .These organisms and those acquired from family members, tend to be community derived antibiotic sensitive organisms, of limited pathogenicity.

In the postnatal ward, the baby acquire organisms from the ward environment, other babies and the clinical staff.

Colonization of the intestinal tract:

The predominant organisms acquired from normal babies are members of the family Enterobacteriaceae(incuding *E.coli, Klebsiella spp, and Citrobacter spp*).

Colonization of the respiratory tract:

Colonization of the upper respiratory tract occurs rapidly and 90% of infants have positive pharyngeal cultures by the third day. *Coagulase*

negative staphylococci are the commonest, followed by *Streptococcus viridians* and *staphylococcus aureus.*

Colonization of the skin:

Skin colonization is very quick, with the number of bacteria increasing 100 fold during the first week. *Coagulase negative staphylococci* predominate but *Staphylococcus aureus* may be found in 65% of infants. A host of other organisms can be found, including yeasts and a range of saprophytic bacteria. The umbilicus and perineum and axilla are the most heavily colonized[90-91].

Colonization of the infants on a Neonatal Intensive Care Units (NICU)

These babies are at greatest risk of becoming colonized by the pathogens, which often show resistance to antibiotics. The pattern of bowel colonization is very different among sick preterm infants. CONS and antibiotic resistant gram negative organism are the predominate organisms[17] [18]

Skin colonization on the NICU is mainly the CONS, which can be isolated from over 90% of all positive culture. *Staphlococcus epidermidis* accounts for 80% and *Staphylococus haemolyticus* for almost all of the remainder**[19]**.

Balance between colonization and infection:

- Most babies become colonized without becoming infected , but in others various host factors or the pathogenicity of the organisms result in tissue invasion and sepsis.

- Intact skin and mucous membranes present an important barrier to microorganisms. Abrasions and cuts, mucosal injury, cannulae, catheter and endotracheal tubes all open the way for bacterial invasion.

- Certain infective agents have an inherent ability to penetrate the placental barrier, often damaging the placenta. Ascent of the vaginal organisms into the uterine cavity prior to rupture of membranes is rare. Once the membranes rupture, the risk increases progressively with time.

- Vaginal delivery inevitably results in contamination and the beginning of colonization of the skin and gut of the baby. Vaginal flora varies considerably from woman to woman and many cases of Early onset sepsis result from the vaginal carriage of opportunistic pathogens.

- In addition to the organism acquired during birth all babies are subject to further microbiological contamination from the environment. People are the main source of such contamination.

- ❖ Babies are less able to combat infection than the older children. This is further compromised by prematurity.

- ❖ Competition between bacteria is a controlling influence on the level of colonization and risk of infection. There is competitive inhibition of gram negative organisms by *Lactobacillus bifidus* in the gut of the breast –fed infants [20].

- ❖ The various risk factors involved in neonatal sepsis are LBW [21], PROM [22], difficult delivery and instrumentation, birth asphyxia, difficult resuscitation [23], ventilator therapy[24], intravenous catheterization[23] and administration of parenteral nutrition[25].

PATHOGENESIS OF SEPSIS

Infection begins when microorganisms circumvent or penetrate host barriers such as skin and mucosa . Depending on the infecting agents , virulence and immuno competence of the patients, local host defences may be overwhelmed , resulting in invasion of the bloodstream.

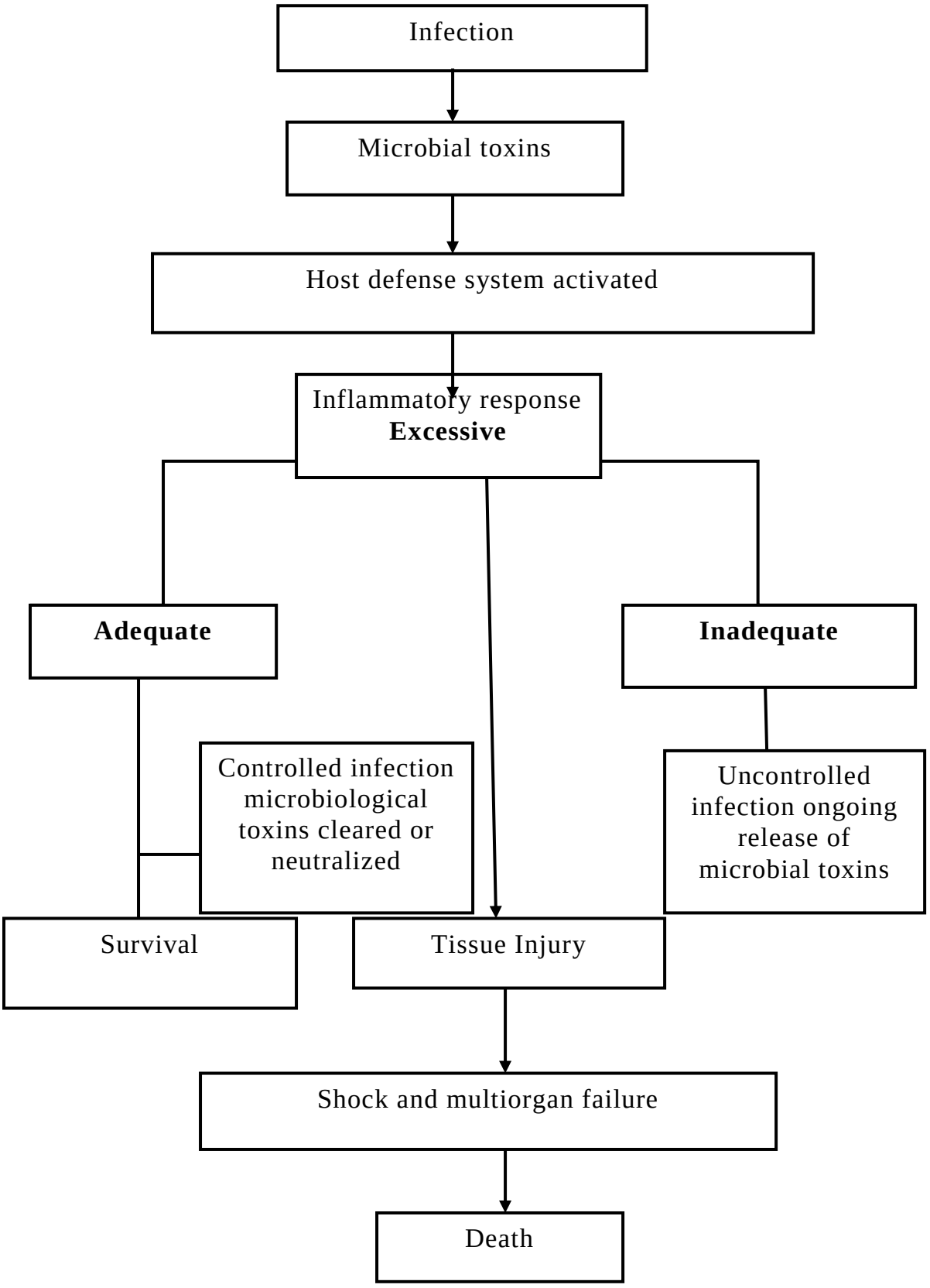
The physiologic manifestations of the inflammatory response are mediated by a variety of pro inflammatory cytokines, principally Tumour necrosis factor (TNF), Interleukin -1 (IL-1) and IL-6 and by products of activation of the complement and coagulation systems. Elevated levels of IL-6, TNF and Platelet activating factor have been

reported in new born infants with neonatal sepsis. IL-6 is the cytokine most often elevated in neonatal sepsis.

Toxic products released by the microbes in the circulation activate systemic host defences, including plasma factors (complement and clotting cascades) and cellular components(neutrophils, monocytes, macrophages and endothelial cells). In turn, activated cells produce potentially toxic mediators that augment the inflammatory response. This escalating immune response in concert with microbial toxins can lead to shock, multiple organ failure ,death. [26]

A predominance of male infants apparent in almost all studies of sepsis in the new born but not among infants in utero. The usual male predominance in neonatal sepsis has suggested the possibility of a sex linked factor in host susceptibility. A gene located on the X chromosome and involved with function of the thymus or with synthesis of immunoglobulins has been postulated. The female has double the number of genes affecting these factors and thus might possess a greater resistance to infection.

PATHOGENESIS OF SEPSIS



ETIOLOGICAL AGENTS

GRAM POSITIVE ORGANISMS

Staphylococcus aureus

Staphylococcus epidermidis.

Streptococcus agalactiae[22]

Listeria monocytogenes[27]

- Through the years, there has been a shift in the microorganisms responsible for neonatal Septicaemia.

- During the 1930's Group A *Streptococcus* were the predominant organisms.

- In the 1950s the phase group I *Staphylococcus aureus* was the most common bacterial agent causing septicaemia in neonatal units. More recently, coagulase positive *Staphlyococcus* disease in nurseries has been caused by organisms of the phase II group. The incidence of CONS sepsis is high in neonatal intensive care units[9][105-106].

- Methicillin –resistant organisms are an increasing problem in NICU neonates and require treatment with vancomycin . MRSA can spread within NICU in epidemic and endemic fashion. Infection control measures including identification of colonized

infants by routine surveillance and cohorting and isolation of colonized infants may be required to prevent spread and persistence of the organisms[18].

- The Group B *Streptococcus* is the most common Gram positive organism causing septicaemia during the first month of life. Vertical transmissions from mother to infants are the most common route of infection. The incidence of Group B *streptococcal* disease has varied widely from place to place and from year to year. Group A *Streptococcal* disease is not as common now as in previous decades. Group D *Streptococci* include the *Enterococci* and several particularly *S.bovis*, which have been found in neonatal infection.

- Coagulase Negative *Staphylococci* may be identified in blood cultures of babies and are frequently dismissed as contaminants. Repeated isolation of the organism from blood associated with clinical signs of septicaemia should alert the physician to its pathogenic role Coagulase negative *Staphylococci* are frequently, but not always associated with arterial or venous catheters or ventriculo peritoneal shunts. Eradication of infection requires removal of the catheter or prosthesis as well as appropriate antibiotic therapy[13] [18].

GRAM NEGATIVE ORGANISMS

Klebsiella pneumoniae.

Escherichia coli.

Pseudomonas aeruginosa.

Haemophilus influenza.

Acinetobacter spp.

Enterobacter aerogenes[28]

Sepsis with the gram negative organisms increasing now a days particularly in the asian countries[29] [30]

- From the late 1950's onwards *E. Coli* has been an important cause of neonatal sepsis. Finally *Klebsiella* have been relatively recent pathogen, accounting for a high proportion of anitibiotic-resistant organisms that colonize and infect babies in neonatal intensive care units[5] . The inadvertent use of broad spectrum antibiotics has led to the emergence of multidrug resistant gram negative bacteria[31]. *Klebsiella* species are of importance in this regard. [32]

- The prevalence rates for a specific bacterial pathogen vary from nursery to nursery and may change abruptly in any one unit.

- The number of nosocomial infections has increased dramatically in the past decade but pathogenic *E.coli, Klebsiella, Enterobacter* and *Pseudomonas* organisms have been responsible for majority of the gram negative infections. GNB causes more than 50% of hospital acquired infections. Most studies have assumed that GNB are part of the transient flora only and easily removed by hygienic hand washing with soap and water[33].

- *E. coli* are the most common gram negative bacteria causing septicaemia during the neonatal period. Approximately 40% of *E. Coli* strains causing septicaemia possess K 1 capsular antigen [34] and strains identical with that in blood can usually be identified in the patient's nasopharynx or rectal cultures[35]. K1 strains in the blood or cerebrospinal fluid of most infants with meningitis related to E. coli. These strains were also cultured from the blood of some infants and adults with sepsis but without meningitis.

- *Pseudomonas aeruginosa* is usually a cause of late onset disease in infants who are presumably infected from their gut flora or from equipment, aqueous solutions ,or on occasion, the hands of health care personnel[34].

- The neonates who receives broad spectrum antibiotics while in an environment potentially contaminated by "water-bugs:

(respirators, moist oxygen) is likely to develop disease caused by *Pseudomonas* species. [34]

- Among the *Enterobacter* species *Enterobacter aerogens, E. cloacae, E. sakazakii* and *E.bormaechei* have caused sepsis and a severe form of necrotizing meningitis in neonates. Contaminated infant formula has been identified as a source of infection. Parenteral nutrition and bladder catheterization are identified as risk factors.

- *Citrobacter* is an occasional inhabitant of GIT and are responsible for disease in neonates and immunocompromised patients. It causes sporadic and epidemic neonatal sepsis and meningitis[14].

- *Acinetobacter species* is an important emerging nosocomical pathogen in neonatal septicemia.[36] [37]Septicaemia due to *Acinetobacter spp* are common in babies with predisposing factors like endotracheal intubation. [29]

- *Neisseria meningitides, Haemophilus influenza* and *Sretptococcus pneumoniae* are relatively uncommon in neonates. *S.pneumoniae* cause early onset sepsis that mimic neonatal disease caused by group*B. Streptococci*. Most people exposed to *Listeria monocytogenes* do not develop illness, but the pregnant women may suffer pregnancy loss and the neonate may develop sepsis and meningitis.

- Inhalation of infected amniotic fluid may produce pneumonia and sepsis in utero, manifested by fetal distress or neonatal asphyxia. Exposure to pathogens at delivery and in the nursery or community is the mechanism of infection after birth.

MULTIDRUG RESISTANT GRAM NEGATIVE BACTERIA

- In septicemic neonates, positivity of blood culture exhibits predominantly multidrug resistant gram negative rods among the isolates. [20]

- Multi drug resistant gram negative bacteria are associated with production of Extended Spectrum Beta Lactamase(ESBL).As these enzymes are active against narrow spectrum cephalosporins, Extended spectrum cephalosporins, aztreonam, the treatment becomes more problematic. [38]

- Several outbreaks of infection caused by *Klebsiella* isolates that are simultaneously resistant to broad spectrum beta lactam antibiotics [39]. Studies carried out in various parts of India have reported prevalence of ESBL producing *Klebsiella* isolates. [40]

- Jain et al observed ESBL production in 87.2% isolates of *Klebsiella species*, 65.3% isolates of *Escherichia coli* and 33.3% isolates of *Pseudomonas aeruginosa*. [41]

CLASSIFICATION OF NEONATAL SEPSIS[6] [12]:

Neonatal sepsis can be classified into **two** major categories depending up on the onset of symptoms.

1.**Early onset of sepsis (EOS)** presents within the first 72 hours of life. In severe cases, the neonate may be symptomatic at birth. Infants with EOS usually present with respiratory distress and pneumonia. The source of infection is generally the maternal genital tract.

RISK FACTORS FOR EOS

Some material / perinatal conditions have been associated with an increased risk of EOS. Knowledge about these potential risk factors would help in early diagnosis of sepsis. Based on the studies from India, the following risk factors seem to be associated with an increased risk of early onset sepsis[10].

- Low birth weight (<2500gms) or prematurity
- Febrile illness in the mother with evidence of bacterial infection within 2 weeks prior to delivery
- Foul smelling and /or meconium stained liquor
- Rupture of membranes >24 hrs
- Prolonged labor(sum of 1^{st} and 2^{nd} stage of labour $\geq$ 24 hrs)
- Perinatal asphyxia(Apgar slore <4at 1 minute)

Presence of foul smelling liquor or **three** of the mentioned risk factors warrant initiation of antibiotic treatment. Infants with **two** risk factors should be investigated and then treated accordingly.

2. Late onset sepsis (LOS) presents after 72hours of age. The source of infection in LOS is either nosocomial (hospital acquired) or community acquired and neonates usually present with septicaemia, pneumonia or meningitis[42].

RISK FACTORS FOR LOS

Various factors that predispose to an increased risk of nosocomial sepsis include

- Low birth weight
- Prematurity
- Admission in intensive care unit
- Mechanical ventilation
- Invasive procedures, administration of parenteral fluids, and use of stock solutions.
- Risk of community- acquired Late onset sepsis
- Poor hygiene
- Poor cord care
- Bottle feeding, and pre lacteal feeds.

Breastfeeding helps in prevention of infections. LOS is a frequent and important problem among VLBW preterm infants.

Successful strategies to decrease Late onset sepsis should decrease Very Low Birth Weight mortality rates, shorten hospital stay and reduce costs[43].

CLINICAL FEATURES

Nonspecific Features

The earlier signs of sepsis are often subtle and nonspecific; indeed a high index of suspicion is needed for early diagnosis. Neonates with sepsis may present with one or more of the following symptoms and signs such as

- Hypothermia or fever,
- Lethargy,
- Poor cry
- Refusal to suck,
- Poor perfusion, prolonged capillary refill time,
- Hypotonia , absent neonatal reflexes,
- Brady / Tachycardia ,
- Respiratory distress,
- Apnea and gasping respiration.
- Hypo/ Hyperglycemia and Metabolic acidosis.

OTHER SYSTEMS INVOLVEMENT

Central Nervous System (CNS)

- Bulging anterior fontanelle,
- Vacant stare,
- High-pitched cry, excess irritability,
- Stupor/coma, seizures, neck retraction.

Presence of these features should raise a clinical suspicion of meningitis

Cardiac System

- Hypotension, poor perfusion, shock

Gastrointestinal System

- Feed intolerance.
- Vomiting, diarrhea.
- Abdominal distension.
- Paralytic ileus
- Neonatal necrotizing enterocolitis (NNEC) [87]

Hepatic System

- Hepatomegaly,
- direct hyperbilirubinemia (especially with urinary tract infections)

Renal System

- Acute renal failure

Hematological

- Bleeding, petechiae, purpura

Skin Changes

- Multiple pustules, abscess, sclerema, mottling, umbilical redness and discharge.

INVESTIGATIONS

Since treatment should be initiated in a neonate suspected to have sepsis without any delay, only minimal and rapid investigations should be undertaken [14]

Blood Culture: Blood culture is the gold standard for diagnosis of septicaemia and should be performed in all cases of suspected sepsis prior to starting antibiotics. It is not safe to take large samples of blood from children. A positive blood culture with sensitivity of the isolated organism is the best guide to antimicrobial therapy. Therefore it is very important to follow the proper procedure for collecting a blood culture. The optimal volume of blood required for successful identification of organism from infants and children has not been clearly delineated [1].

Baron EJ et al., stated that recommendations for blood volumes for cultures from infants and children, only 1-5 ml of blood should be drawn for bacterial culture.

A positive blood culture with sensitivity of the isolated organism is the best guide to antimicrobial therapy. Therefore it is very important to follow the proper procedure for collecting a blood culture.

It is now possible to detect bacterial growth within 12-24 hours by using improved bacteriological techniques such as BACTEC and BACT/ALERT blood culture systems. These advanced techniques can detect bacteria at a concentration of 1-2 colony-forming unit (cfu) per mL.

Septic Screen

All neonates suspected to have sepsis should have a septic screen to corroborate the diagnosis [6] [10]. However, the decision to start antibiotics need not be conditional to the sepsis screen result, if there is a strong clinical suspicion of sepsis. The various components of the septic screen include

1) Total leukocyte count (TLC)

2) Absolute Neutrophil count (ANC)

3) Immature to total (IT) neutrophil ratio

4) Micro-erythrocyte sedimentation rate

5) C -reactive protein (CRP) :

- The ANC varies considerably in the immediate neonatal period[42].
- The lower limit for normal ANC begins at 1800/cmm at birth, rises to 7200/cmm at 12 hours of age and then declines and persists at 1800/cmm after 72 hours of life.
- The I/T ratio is lesser than or equal to 0.16 at birth and declines to a peak value of 0.12 after 72 hours of age
- Presence of two abnormal parameters in a screen is associated with a sensitivity of 93-100%, specificity of 83%, positive and negative predictive values of 27% and 100% respectively in detecting sepsis. Hence, if two (or more) parameters are abnormal, it should be considered as a positive screen and the neonate should be started on antibiotics.
- If the screen is negative but clinical suspicion persists, it should be repeated within 12 hours. If the screen is still negative, sepsis can be excluded with reasonable certainty.

Lumbar puncture (LP)

❖ The incidence of meningitis in neonatal sepsis has varied from 0.3-3% in various studies[4][9]. The clinical features of septicemia

and meningitis often overlap; it is quite possible to have meningitis along with septicemia *without* any specific symptomatology. In Early Onset Sepsis, lumbar puncture is indicated in the presence of a positive blood culture or if the clinical picture is consistent with septicaemia.

- It is not indicated if antibiotics have been started solely due to the presence of risk factors. In situations of late onset sepsis, LP should be done in all infants prior to starting antibiotics.

- Lumbar puncture could be postponed in a critically sick neonate. It should be performed once the clinical condition stabilizes. The cerebrospinal fluid characteristics are unique in the newborn period .

RADIOLOGY

- Chest x-ray should be considered in the presence of respiratory distress or apnea.

- An abdominal x-ray is indicated in the presence of abdominal signs suggestive of Necrotizing Enterocolitis (NEC)

- Neurosonogram and computed tomography (CT scan) should be performed in all patients diagnosed to have meningitis.

URINE CULTURE

- Urine cultures have a low yield and are not indicated routinely. However, neonates at risk for fungal sepsis, with urogenital malformation or vesicoureteral reflex or suspected of UTI (crying during micturition) should have a urine examination done to exclude urinary tract infection (UTI).

- Urine cultures obtained by suprapubic puncture, bladder catheterization or clean catch sample from midstream of urine

- UTI may be diagnosed in the presence of one of the following:

  - >10 WBC/mm3 in a 10 mL centrifuged sample
  - >104 organisms/mL in urine obtained by catheterization and
  - any organism in urine obtained by suprapubic aspiration

ACUTE PHASE PROTEINS

C-REACTIVE PROTEIN

C-reactive protein is a nonspecific acute phase protein that rises in response to infectious and noninfectious inflammatory processes. It is synthesized by hepatocytes. Good evidence exists to support the use of CRP measurement along with other established diagnostic tests (Blood culture,WBC count and Differential count) to establish or exclude the diagnosis of sepsis in full term or near term infants.

The three clinical laboratory methods namely

1) Qualitative test

2) Semi-quantitative test

3) Quantitative test are carried out to measure serum CRP levels.

- The **quantitative immunoassay** is the most rapid sophisticated sensitive method of detecting and measuring CRP. Accurate measurement of CRP can be made by laser nephelometry or single radio immuno diffusion assay[44].

- Increased CRP levels can occur in infants for upto three days of life from non-infectious causes. **Maternal fever, PROM, Fetal distress, Pneumothorax and Meconium aspiration pneumonitis** could cause elevated CRP levels.

- The CRP levels do not always rise above 10mg/ l in preterm infants or those infants with overwhelming sepsis, resulting in a false negative test results[51][52].

- In a study done by Khassawneh et al.,[53] (2007), it was concluded that CRP ,IL-6 and IgM are useful in the diagnosis of gram negative neonatal sepsis and CRP continues to be the best single test. According to them a CRP value 5 mg/L was the best among the three parameters with 95% sensitivity.

- According to Philip A and Andrews P [54](1986) the semi quantitative latex agglutination assay involves the use of serial dilution of serum with saline.

- As per Murex [55] (1995) diagnostics, Murex CRO the qualitative latex agglutination test can be performed rapidly at the bedside within 10 to 15 minutes. CRP is a useful serum marker to assess and monitor the presence, severity and course of the inflammatory response in infectious and non-infectious disorders including acute myocardial infarction, angina, malignancies, burns and trauma as reported by Jaye D and Waites K [56] (1997).

PROCALCITONIN:

Procalcitonin is a 116 aminoacid peptide that has an approximate MW of 14.5 KDa and belongs to the calcitonin super family of peptides.

❖ Its level increases significantly in severe systemic infections, as compared to other parameters of microbial infection.

❖ The short half –life (25-30 hours in plasma) of PCT, coupled with its virtual absence in health and specifically for bacterial infections, gives it a clear advantage over the other markers of bacterial infection.

- Studies have also shown that an increase in PCT levels is minimal in viral infections while levels increase rapidly after a single injection with endotoxin. Increased PCT levels comparable to what is observed in severe sepsis were also seen in Addisonian crisis, transplant patients receiving T-cells antibody therapy[45].

- PCT is physiologically elevated during first 3 days of life. But it has been found to be a reliable marker of LOS in newborn babies with a sensitivity and specificity of nearly 100%. Comparative studies have shown that PCT is a more reliable marker of sepsis compared to CRP.

- Quantitative measurement of PCT is performed by immnuoluminometric assay (ILMA) with two monoclonal antibodies. After the initial surge of PCT during first 3 days of life, the mean normal serum PCT is around 0.5 ng/ml[46].

- Procalcitonin is a biomarker that exhibits greater specificity than other pro inflammatory marker (e.g Cytokines) in identifying patients with sepsis and can be used in the diagnosis of bacterial infection.

- Resch et al[48] (2003)have documented that the reliability of Procalcitonin determination in the diagnosis of early onset neonatal sepsis as compared to CRP and IL-6 revealed sensitivity of PCT as 77% compared to 54% and 69% for IL-6 and CRP.

- Carrigan et al.,[49] (2004) reported that the broth culture method is the gold standard for the diagnosis of bacterial infection but a definitive result can take 24 hrs or more before a conclusive diagnosis. Procalcitonin owing its specificity to bacterial infection has been proposed as a pertinent marker in the rapid diagnosis of bacterial infections. Multivariate logistic regression identified that a PCT=2.6 ng / ml was independently associated with the development of sepsis shock.

- Simon et al., [50](2004) have stated that Procalcitonin level increases significantly in severe systemic infections as compared to other parameters of microbial infection.

The incidence of meningitis in neonatal sepsis has varied from 0.3-3% in various studies. Lumbar puncture is indicated in the presence of a positive blood culture or if the clinical picture is consistent with septicemia.

In early onset sepsis, urine cultures have a low yield and are not indicated. Urine cultures obtained by suprapubic puncture or bladder catheterization have been recommended in all cases of LOS.

Adequate and proper supportive care is crucial in a sick neonate with sepsis. He/ she should be nursed in a thermo-neutral environment taking care to avoid hypo/hyperthermia. Oxygen saturation should be maintained in the normal range; mechanical ventilation may have to be

initiated if necessary. If the infant is hemo dynamically unstable, intravenous fluids should be administered and the infant is to be monitored for hypo/hyperthermia. There cannot be a single recommendation for the antibiotic regimen of neonatal sepsis for all settings.

The choice of antibiotics depends on the prevailing flora in the given unit and their antimicrobial sensitivity. Decision to start antibiotics is based upon the clinical features and / or a positive septic screen. However duration of antibiotic therapy is dependent upon the presence of a positive blood culture and meningitis.

Aggressive management of suspected maternal chorioamnionitis with antibiotic therapy during labor along with rapid delivery of the infant reduces the risk of early- onset neonatal sepsis. Vertical transmission of Group B *Streptococcal* sepsis is significantly reduced by selective intrapartum chemoprophylaxis.

Principles for the prevention of nosocomial infection include adherence to universal precautions with all patient contact, avoiding nursery crowding, strict compliance with hand washing , meticulous neonatal skin care, minimizing the risk of catheter contamination, decreasing the number of veni-punctures and mechanical ventilation days and providing education and feedback to nursery personnel. Management

SUPPORTIVE

- Adequate and proper supportive care is crucial in a sick neonate with sepsis. He/she should be nursed in a thermo-neutral environment taking care to avoid hypo/hyperthermia

- Oxygen saturation should be maintained in the normal range; mechanical ventilation may have to be initiated if necessary.

- If the infant is hemodynamically unstable, intravenous fluids should be administered and the infant is to be monitored for hypo/hyperglycemia. Volume expansion with crystalloids/colloids and judicious use of inotropes are essential to maintain normal tissue perfusion and blood pressure.

- Packed red cells and fresh frozen plasma might have to be used in the event of anemia or bleeding diathesis.

ANTIMICROBIAL THERAPY

Indications for starting antibiotics: The indications for starting antibiotics in neonates at risk of EOS include any one of the following:

a. Presence of >3 risk factors for early onset sepsis (see above)

b. Presence of foul smelling liquor

c. Presence of more than 2 antenatal risk factor(s) and a positive septic screen and

d. Strong clinical suspicion of sepsis.

The indications for starting antibiotics in LOS include:

a. Positive septic screen and/or

b. Strong clinical suspicion of sepsis.

CHOICE OF ANTIBIOTICS

Empirical antibiotic therapy should be unit-specific and determined by the prevalent spectrum of etiological agents and their antibiotic sensitivity pattern. Antibiotics once started should be modified according to the sensitivity reports.

1) The choice of antibiotics depends on the prevailing flora in the given unit and their antimicrobial sensitivity. Decision to start antibiotics is based upon clinical features and/ or a positive septic screen. However duration of antibiotic therapy is dependent upon the presence of a positive blood culture and meningitis.

2) For infections that are likely to be community-acquired where resistant strains are unlikely, a combination of ampicillin or penicillin with gentamicin may be a good choice as a first line therapy.

3) For infections that are acquired during hospital stay, resistant pathogens are likely and a combination of ampicillin or cloxacillin with gentamicin or amikacin may be instituted. In

nurseries where this combination is ineffective due to the presence of multiple resistant strains of *klebsiella* and other gram-negative bacilli, a combination of a third generation cephalosporin (cefotaxime or ceftazidime) with amikacin may be appropriate[58][59].

4) For sepsis due to *enterococcus*, a combination of ampicillin and gentamicin is a good choice for initial therapy. Vancomycin should be used for the treatment of enterococcus resistant to the first line of therapy.

- Gladstone et al., [57] (1990) documented the introduction of aminoglycoside antibiotics- Kanamycin in the early 1960s and later Gentamycin provides vastly improved therapy for bacteremia due to Gram negative organisms, the leading cause of sepsis at that time.

- Benzyl Penicillin is most suitable for the treatment of infections due to Group B Streptococci.

- Septicemia due to *Pseudomonas aeruginosa* is best managed by ceftazidime.

- Betalactamase resistant penicillins and vancomycin are indicated in the treatment of infection caused by Staphylococcus aureus.

- Ampicillin is the drug of choice for Listeriosis.

- A combination of amikacin and cefotaxime is ideal for treatment of neonatal meningitis (Meharbansingh 2010).

PROGNOSIS

The reported mortality rate in neonatal sepsis from various study in India between 10 to 15 % .Early institution of specific antibiotic therapy with the aid of blood culture with sepsis screen excellent supportive care close monitoring of vital signs and judicious use of fresh blood , fresh frozen plasma and immunotherapy is slightly to improve the outcome of the neonates with septicemia[60]

MATERIALS AND METHODS

Study Place: This study was conducted in the Institute of Microbiology, Madras Medical College, Rajiv Gandhi Government General Hospital (RGGGH), Chennai in association with Neonatal unit Institute of Child Health, Egmore. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were included in this study.

Study Design: Cross sectional study

Study Period: October 2014 to August 2015

Study Group: A total of 100 clinically suspected cases of newborns admitted in newborn ward with symptoms and signs suggestive of sepsis were taken for the study.

ETHICS CONSIDEATION

This study was approved by the institutional scientific and ethical committee and written informed consents were obtained from the parents. Neonatal history was taken from the parents by structured questionnaire.

INCLUSION CRITERIA

The clinical criteria taken as indicative of sepsis were:

1) Maternal risk factor such as fever, PROM >24hrs

2) Neonatal history: Low birth weight (<2500gm), premature birth (<37 weeks)

3) Signs and symptoms of sepsis: Feeding intolerance, lethargy, temperature instability, apnea, respiratory distress poor perfusion, seizures, tachypnea, bradycardia, abdominal distension or vomiting.

Neonates who had any features mentioned from I and II associated with two or more clinical symptoms of sepsis would warrant a septic screen.

According to clinical symptoms of sepsis, microbiologic and laboratory results(C Reactive Protein, white cell count, platelet count and blood culture), neonates classified into three groups.

- Proven sepsis: Positive blood culture and clinical symptoms of sepsis.
- Suspected sepsis: Negative blood culture with abnormal laboratory results.
- Clinical sepsis: Negative blood culture and normal laboratory results.

EXCLUSION CRITERIA

- Newborns who were started antibiotics or those who developed the signs of sepsis within 72hours of discontinuation of antibiotics.
- Birth asphyxia, aspiration syndromes
- Inborn errors of metabolism
- Congenital anomalies

SAMPLE COLLECTION

Under strict aseptic precautions, venepuncture site was cleaned with 70% alcohol and then with 2 % Povidone Iodine [61]. The disinfectant was allowed to act for 1 minute and then 1-2ml of blood sample was collected with a sterile syringe and added into a sterile crew capped blood culture bottle containing 10 ml of sterile Brain Heart Infusion broth(BHI broth) at the bed side was labelled with name, age ,sex, IP No, date and time of collection and culture bottles transported immediately to the laboratory.

Using a sterile syringe and needle, withdraw about 1-2 ml for blood culture, another 1-2 ml for measurements of procalcitonin, c-reactive protein.

Serum was separated from blood cells by centrifugation and stored in 2 plastic tubes at -20°C for measurements of PCT and CRP.

SAMPLE PROCESSING

The culture bottle were incubated at 37 °C for 24-48 hours .Inspection and subculture was done during the first day and examined for turbidity, pellicle formation and deposits, the subcultures made onto the following plates using sterile techniques [62].

- MacConkey Agar plate
- 5%Sheep Blood Agar plate
- Chocolate Agar plate

INTERPRETATION

All plates were checked for growth in culture plates after 24-48 hours The colony size, shape, edge, margin and consistency was noted.

IDENTIFICATION

Gram's staining was performed from growth obtained[1].

In case of Gram Positive cocci, the following tests were performed;

- Catalase,
- Slide coagulase,

- Tube coagulase,
- Novobiocin sensitivity,
- Optochin sensitivity,
- Bile esculin hydrolysis,
- Heat test.

ANTIBIOTIC SUSCEPTIBILITY TESTS

Antibiotic susceptibility testing of the bacterial isolates were performed by Kirby Bauer's disk diffusion method on Mueller-Hinton agar and zone diameter were interpreted according to CLSI guidelines[63].

Medium Used: MHA (Mueller Hinton agar plate)

Inoculum: 0.5 McFarland turbidity (lawn culture)

Incubation: 37°C Ambient air, incubated for 16-18 hours

PREPARATION OF INOCULUM FOR SENSITIVITY TESTING

About 4-5 colonies of similar morphology were picked up with the help of straight wire and inoculated in 5ml of suitable broth, and it is incubated for 3-5hrs which gives 0.5 McFarland turbidity. A sterile cotton swab was taken and dipped into it and pressed firmly against the wall of the test tube so as to remove the excess broth from the swab.

Mueller Hinton agar plate were dried and swabbed in three directions to ensure complete distribution of the inoculums over the entire plate. The antimicrobial discs were dispensed on the Mueller Hinton agar plate using dispenser and pressed down to give complete contact with the surface of the agar.

The discs were distributed evenly on the plate and the distance should not be closer than 24mm from centre to centre not more than 6 discs were placed in a single plate [ ]. After an incubation period of 16-18 hrs each plate was examined. The diameter of the zones which gave complete inhibition was measured. The diameter of zones of inhibition was interpreted according to CLSI standards for each organism.

The following standard strains were used.

- Staphylococcus aureus- ATCC 25923
- Escherichia coli- ATCC 25922
- Pseudomonas aeruginosa- ATCC 27823

Panel of antibiotics included for testing antimicrobial sensitivity of Gram positive cocci.

Antibiotics	**Disc Content**	**Zone of Inhibition**		
		Sensitive	**Intermediate**	**Resistance**
Penicillin	10 units	≥29	-	≤28
Erythromycin	15µg	≥23	14-22	≤13

Antibiotics	Disc Content	Zone of Inhibition		
		Sensitive	Intermediate	Resistance
Cefoxitin	30µg	≥22	-	≤21
Ciprofloxacin	5µg	≥21	16-20	≤15
Co-trimoxozole	1.25/23.75µg	≥16	11-15	≤10
Amikacin	30µg	≥17	15-16	≤14
High level gentamicin	120µg	≥10	7-9	≤6
Chloromphenicol	30µg	≥18	13-17	≤12

Stapylococcus aureus strains were screened for Methicillin resistance.

DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS:*

Cefoxitin Disc Method

Colonies of *Staphylococcus aureus*(test isolate) were inoculated in peptone water and matched with 0.5 Mac Farland standard. The suspension was streaked onto cation adjusted Muller-Hinton agar plates. And *Staphylococcus aureus* ATCC 25923 (Control) was cultured onto another separate cation adjusted MHA. Cefoxitin disc (30 µg) were placed on the lawn culture of both the test and control isolates and incubated at 33-35ºC overnight.

INTERPRETATION

As per **CLSI**guidelines,

Zone of inhibition: ≥22mm (mec A negative)

Zone of inhibition: ≤21mm (mec A positive)

Cefoxitin is used as a surrogate marker for mec A- mediated oxacillin resistance. Isolates that test as mec A positive should be reported as oxacillin (not cefoxitin) resistant.

Minimum Inhibitory Concentration (MIC) was performed for MRSA isolates.

The minimum inhibitory concentration is the least amount of antimicrobial that will inhibit visible growth of an organism after overnight incubation.

Minimum Inhibitory Concentration (MIC) for Detecting Vancomycin Resistance

- Culture media: cation adjusted Muller Hinton broth. (pH 7.2-7.4)
- Preparation of stock antibiotic solution[83]:
- Antibiotic stock antibiotic solution was prepared using the formula,

$$\frac{1000}{P} \times V \times C = W$$

Where P = potency of the antibiotic in relation to the base.

V = Volume of the stock solution to be prepared (10ml).

(For vancomycin, P = 950/1000mg; Himedia)

C = Final concentration of the antibiotic solution (1024µg/ml)

W = Weight of the antibiotic to be dissolved in the volume V.

Preparation of Working Antibiotic Solution

10.77mg of vancomycin was weighed and dissolved in 10ml of distilled water. This solution was used as stock solution and stored in a sterile screw capped container.

Preparation of Inoculum for the Test and ATCC Control Isolates

0.1ml of Mac Farland turbidity matched test organism and control was added to 9.9ml of separate MH broth and mixed well and stored in separate sterile screw capped containers.

Procedure

Thirteen sterile tubes were arranged in a rack. Using micropipette, 1ml of peptone water was filled in all the test tubes. Doubling dilution of antibiotic (µg/ml) was prepared. 1ml of stock solution of the antibiotic was added to 1st tube which would be 512 dilution. Then, 1ml was pipetted from the 1sttube and transferred to the second, 128 dilution and then serially diluted to subsequent tubes (64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125). 1ml was discarded from the last tube.

Finally 1ml of inoculum was added to all the thirteen tubes. The tubes were incubated at 35ºC for 18-24hrs. Similar procedure was followed for the control strain also.

Observation

The MIC of ATCC control strain was observed and since it was within sensitive range. In the test isolates the lowest concentration of the antibiotic in there was no visible growth was taken as the MIC of the for the test isolate.

Interpretation

MIC of Vancomycin

≤ 2µg/ml: Susceptible.

4-8µg/ml: Intermediate.

≥16µg/ml: Resistant

Gram Negative Organisms

The following preliminary and standard biochemical tests were performed;

- Catalase,
- Oxidase,
- Motility,

- Methyl red,
- Voges Proskauer,
- Citrate utilization test,
- Urease hydrolysis,
- Hugh-Leifsons OF test,
- Mannitol motility,
- Lysine decarboxylase,
- Ornithine decarboxylase,
- Arginine dihydrolase.
- Glucose, sucrose, lactose, maltose, mannose fermentation tests were also performed.
- Antibiotic susceptibility tests were performed by Kirby Bauer's disk diffusion method on Mueller- Hinton agar plate according to CLSI guidelines.

Panel of antibiotics included for testing antimicrobial sensitivity of Gram negative bacilli

Antibiotics	**Disk content/ Gram negative bacilli**		**Diameter of zone inhibition in mm**		
			Sensitive	**Intermediate**	**Resistant**
Amikacin	30µg		≥17	15-17	≤14
Gentamicin	10µg		≥15	13-14	≤12
Ciprofloxacin	5µg		≥21	8-20	≤17
Cotrimoxozole	1.25/23.75µg		≥16	11-15	≤10
Cefotaxime	30µg	Enterobacteriaceae	≥26	23-25	≤22
		Acinetobacter sp.	≥23	15-22	≤14
Ceftazidime	30µg	Enterobacteriaceae	≥21	18-20	≤17
		P.aeruginosa Acinetobacter sp.	≥18	15-17	≤14
Imipenem	10µg	Enterobacteriaceae	≥23	20-22	≤19
		P.aeruginosa	≥19	16-18	≤15
		Acinetobacter sp.	≥16	14-15	≤13
Piperacilin-Tazobactum	100/10µg		≥21	18-20	≤17

EXTENDED SPECTRUM ß-LACTAMASES (ESBL) DETECTION METHODS[78][79][63][80][84]:

ESBL‟s are classified under Bush class A ß- lactamases which are capable of hydrolyzing penicillins – oxyiminocephalosporins and monobactams (Aztreonam) and inhibited by ß-lactamase inhibitors

(clavulanic acid, sulbactum and tazobactum) but have no detectable activity against cephamycins or carbapenems (Imipenem, Meropenem).

ESBL SCREENING METHOD[83]:

Isolates of gram negative bacilli showing the following resistance pattern were considered to be possible ESBL producing strains.

Antibiotic	Zone diameter for possible ESBL producing strain
Ceftazidime(30µg)	≤22mm
Cefotaxime(30µg)	≤27mm
Ceftriaxone(30µg)	≤25mm
Aztreonam(30µg)	≤27mm

1. Double Disk Diffusion Synergy Test:[81]

In this test discs of third generation cephalosporins and Augmentin [Amoxicilin and Clavulanic acid] (20µg/10µg) (Himedia) were kept 30mm apart from centre to centre on a lawn culture of 0.5 Mcfarlands test lsolate on Mueller Hinton agar (MHA) A clear extension of the edge of the inhibition zone of cephalosporin towards Augmentin disc was interpreted as positive for ESBL production.

2.Phenotypic Confirmatory Double Disk Test: (PCDDT) [82]

Antibiotics used:

1) Ceftazidime (30µg)

2) Ceftazidime/Clavulunic acid (30/10µg)

Detection of Extended spectrum beta lactamase production

Using sterile loop five to six colonies of similar morphology of the test organism were picked up and inoculated in peptone water and incubated at 37ºC for 2-4hrs and then the suspension was matched with 0.5 Mac Farland standard ($1.5x10^8$cfu/ml). Lawn culture was perfomed on MHA plate. Ceftazidime (30 µg), Ceftazidime/clavulanate (30/10 µg) disks were placed on the plate with 24mm gap between each disks and incubated at 37ºC overnight.

Interpretation: (53)

A zone of $\geq$ 5mm around the combination disks than the other counterpart disks is confirmed to be due to ESBL production.

C-REACTIVE PROTEIN ESTIMATION (TURBILATEX METHOD)

Clinical Significance

The estimation of CRP was done by GenX CRP Turbilatex Quantitative method marketed by Proton Biologicals India Pvt. Ltd, Bangalore.

PRINCIPLE OF THE METHOD

The CRP-Turbilatex is a quantitative turbidimetric test for the measurement of C-reactive protein (CRP) in human serum or plasma. Latex particles coated with specific anti-human CRP are agglutinated when mixed with samples containing CRP. The agglutination cause an absorbance change, dependent upon the CRP content of the patient

sample that can be quantified by comparison from a calibrator of known CRP concentration.

REAGENT

Diluent(R1)	Tris buffer 20 mmol/L, Ph 8.2. sodium azide 0.95 g/;
Latex (R2)	Latex particles coated with goat IgG anti-human CRP,
Calibrator	Ph 7.3. sodium azide 0.95 g/L Human serum. C-Reactive Protein concentration is stated on the vial label.

PREPARATION

Working reagent: Swirl the latex vial gently before use. Prepare the necessary amount as follows.

1 ml Latex Reagent +9 ml Diluent

PROCEDURE:

Calibrate into test tubes labelled Calibrator(C) and Test(T).

Reagent	(Calibrator)	(Test Sample)
Working Reagent	0.5 mL	0.5 mL
CRP Calibrator(Conc. 44 mg/L)	5 Ml	-
Sample	-	5 µL

1) Bring the working reagents and the photometer to 37° C.

2) Assay conditions :

a. Wavelength: 540 nm (530-550nm)

b. Temperature : 37° C

c. Cuvette light path : 1cm

3) Adjust the instrument to zero with distilled water.

4) Pipette into a cuvette.

5) Mix and read the absorbance

a. Initial absorbance A1- exactly after 60sec.

b. Final absorbance A2- exactly 60 sec. after A1

CALCULATIONS

$$CRP\ Concentration(mg/L) = \frac{(A2-A1)sample}{(A2-A1)calibrator} \times 44(Calibrator\ concentration)$$

The reading is done by semiautoanalyzer.

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures.

REFERENCES VALUES

Normal values up to 6 to 8 mg/L.

PROCALCITONIN ESTIMATION

The measurement was done by QDx Instacheck PCT Kit manufactured by Biotech Med incorporated, Korea.

PRINCIPLE

The test uses a sandwich immune detection method, such that the detector antibody in buffer binds to PCT in serum sample and antigen – antibody complexes are captured to another PCT antibody that has been immobilized on test strip as sample mixture migrates nitrocellulose matrix. Thus the more PCT antigen in serum, the more antigen-antibody complexes accumulated on the test strip. Signal intensity of fluorescence on detector antibody reflects the amount of antigen captured and is processed by QDx Instacheck ^{TM} Reader to show PCT concentration in specimen. The working range of QDx Instacheck ^{TM} PCT test is 0.25 ~ 100ng/mL.

DIAGNOSIS OF BACTERIAL INFECTION/SEPSIS

PCT<0.5	Local bacterial infection is possible
0.5<PCT<10	Infection is possible
2<PCT<10	Infection(sepsis) is likely, unless other cause are known
PCT>10	Severe bacterial sepsis or septic shock

PROCEDURE

1) 150µL of the serum or plasma sample was transferred using a pipette to the tube containing the detection buffer.

2) The lid of the detection buffer tube was closed and mixed the sample thoroughly with the detection buffer by shaking the tube about 10 times.

3) 75µL of sample mixture from the detection buffer tube was pipetted out and dispensed it into the sample well on the test cartridge.

4) For scanning the sample, loaded test cartridge was inserted into the test cartridge holder of the QDx Instacheck Reader.

5) QDx Instacheck Reader started scanning after 12mins

6) The test result was displayed on the screen.

INTERPRETATION OF TEST RESULT

- QDx Instacheck Reader calculates the test result automatically and displays PCT concentration of the test sample in terms of ng/mL.
- Working range of QDx Instacheck PCT is 0.25-100 ng/mL
- Cut off of QDx Instacheck PCT is 0.5 ng/mL
- Test result of more than 2ng/mL may reflect a severe sepsis.

QUALITY CONTROL

- Control standards are not provided with QDx Instacheck PCT.

- Internal control: QDx Instacheck PCT test has an inbuilt quality control indicator that satisfies the routine quality control requirements. This internal control test is performed automatically each time a clinical sample is tested.

- An invalid result from the internal control leads to display an error message on the QDx Instacheck Reader indicating that the test should be repeated.

RESULTS

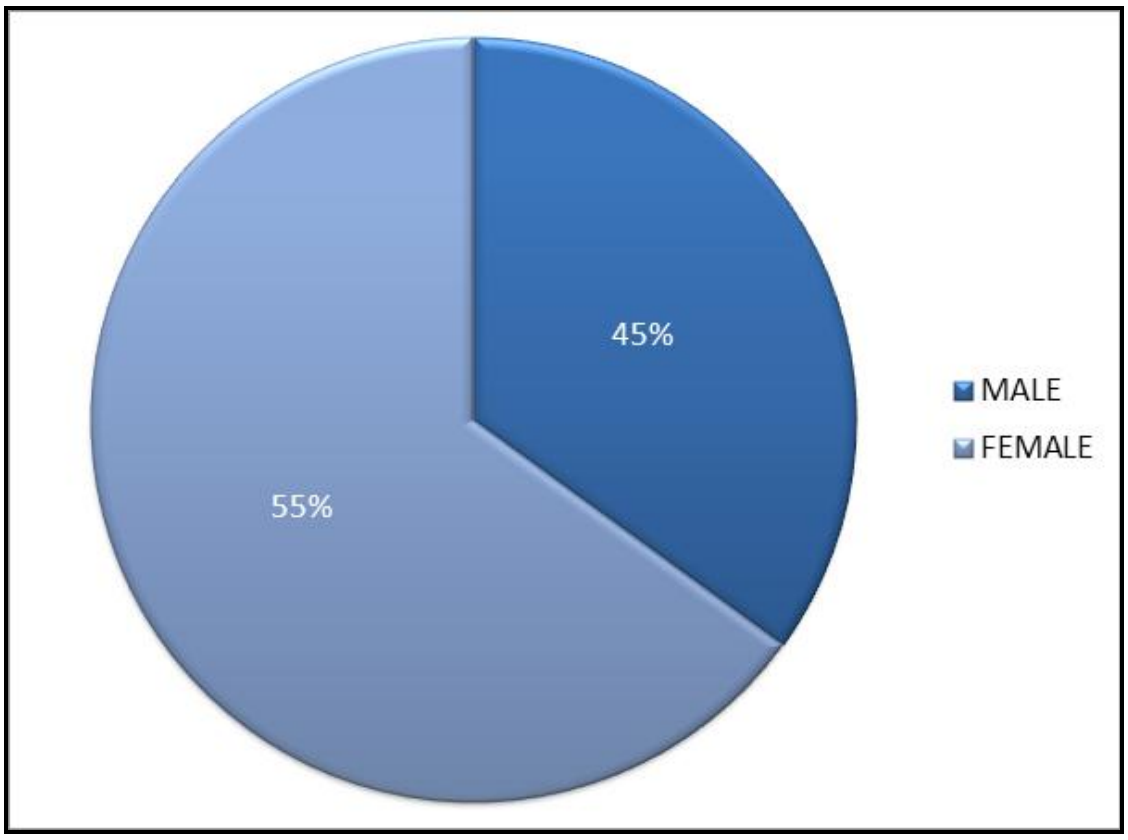
This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai in association with the department of Neonatology, at the Institute of Child Health, Egmore.

Total number of 100 newborns with signs and symptoms of sepsis (who satisfied the inclusion criteria) were included in this study from october 2014 to August 2015.

TABLE-1: ANALYSIS OF SEX DISTRIBUTION IN NEONATAL SEPSIS CASES (N=100)

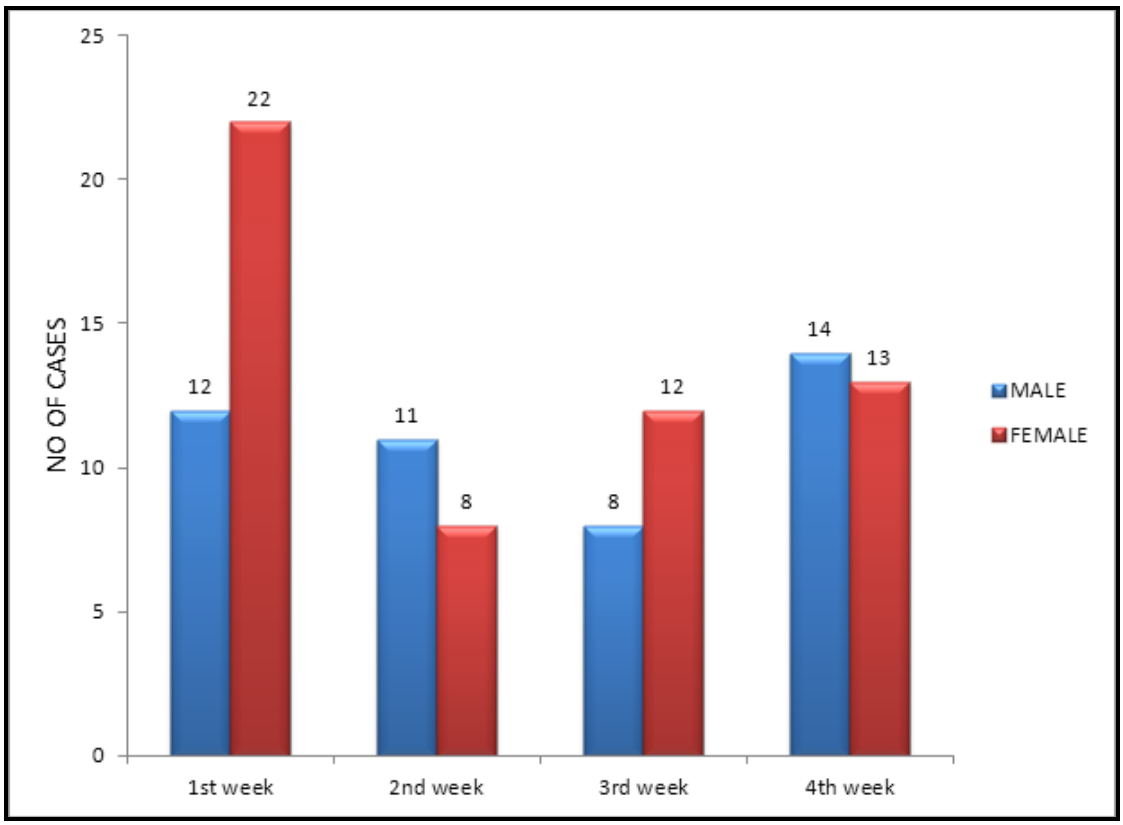
Gender	Number of cases(%)
Male	45(45%)
Female	55(55%)

FIGURE-1: ANALYSIS OF SEX DISTRIBUTION IN NEONATAL SEPSIS CASES (N=100)



Among 100 newborns, 45% were male babies and 55% were females babies. Females were predominantly affected groups than male.

FIGURE 2 : TIME OF CLINICAL PRESENTATION IN 28 DAYS



Majority (34%) of the babies affected during the first week (early neonatal period). Analyzing this data, overall females were most commonly affected in neonatal period.

TABLE 2: DISTRIBUTION OF NEONATAL SEPSIS CASES ACCORDING TO THE ONSET (N=100)

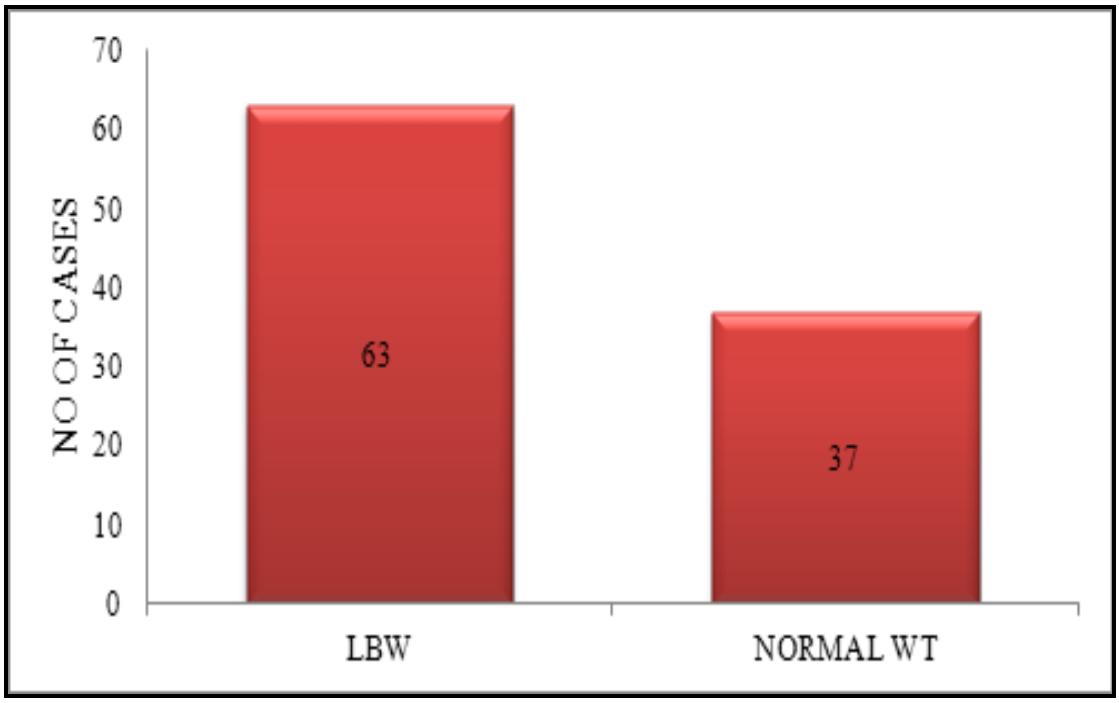
Onset	Male	Female
	Number(%)	Number(%)
Early Onset sepsis (EOS). (<72hrs)	5(33.3%)	10(66.7%)
Late Onset Sepsis (LOS).(>72hrs)	40(47.1%)	45(52.9%)

It was found that 15% of babies had early onset sepsis and 85% of the babies had late onset sepsis. The incidence of late onset sepsis was higher than the early onset sepsis in the present study.

TABLE 3: WEIGHTWISE DISTRIBUTION OF NEONATAL SEPSIS CASES (N=100)

Birth weight	Male	Female
	Number(%)	Number (%)
LBW	22(35%)	41(65%)
Normal Weight	23(62.2%)	14(37.8%)

FIGURE 3: ANALYSIS OF WEIGHTWISE DISTRIBUTION OF NEONATAL SEPSIS CASES (N=100)

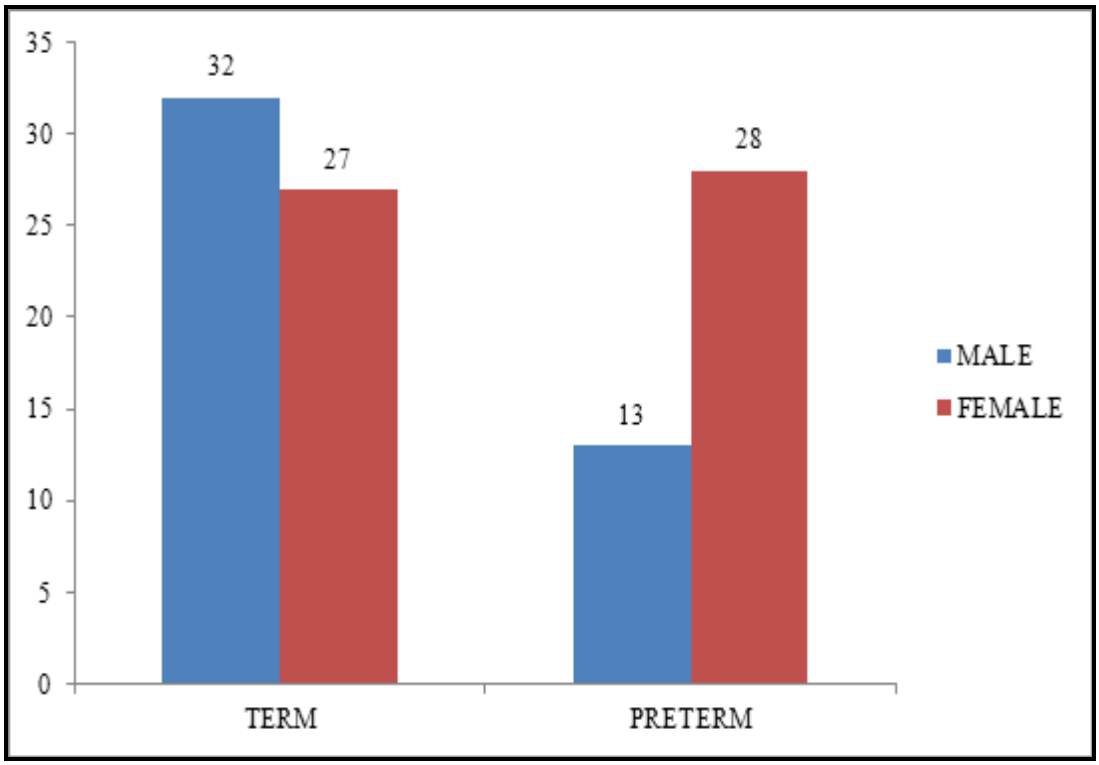


Out of 100 cases studied for neonatal septicemia, 63% of the neonates were low birth weight (< 2.5 kg). Birth weight above 2.5kg accounted for 37% of neonatal septicemia. It was found that low birth weight babies were more prone for neonatal sepsis.

TABLE 4: DISTRIBUTION OF CASES ACCORDING TO PERIOD OF GESTATION (N=100)

Gender	No of Term Cases (%)	No of Preterm Cases (%)
Male	32(54.2%)	13(31.7%)
Female	27(45.8%)	28(68.3%)

FIGURE-4: DISTRIBUTION OF CASES ACCORDING TO PERIOD OF GESTATION (N=100)



Among Term cases (59), male babies (54.2%) were affected.

TABLE 5: RISK FACTORS ASSOCIATED WITH NEONATAL SEPSIS (N=100)

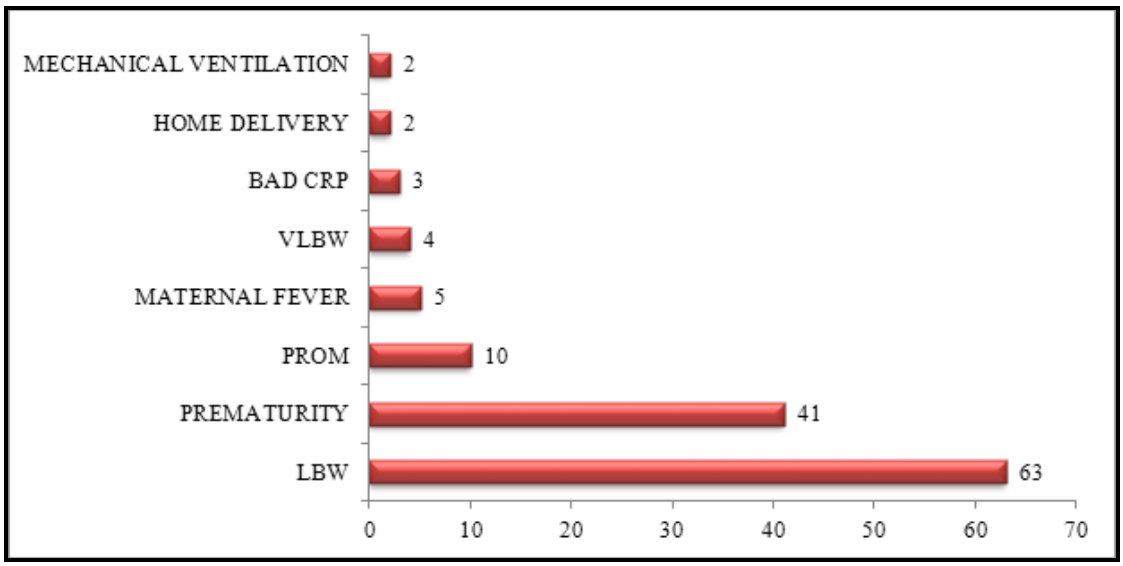
Risk Factor	Number of cases
LBW	63(63%)
PREMATURITY	41(41%)
PROM	10(10%)
MATERNAL FEVER	5(5%)
VLBW	4(4%)
BAD CRP	3(3%)
HOME DELIVERY	2(2%)
MECHANICAL VENTILATION	2(2%)

CRP-Child Rearing Practices

PROM-Premature Rupture of Membrane

VLBW-Very Low Birth Weight

FIGURE 5: RISK FACTORS ASSOCIATED WITH NEONATAL SEPSIS (N=100)



The risk factors associated with an increased risk of neonatal sepsis among 100 cases, the commonest risk factor was low birth weight (63%) and prematurity (41%)

TABLE 6: CLINICAL FEATURES OF NEONATES WITH SEPTICEMIA (N=100)

S. No	Clinical Features	Number (%)
1	REFUSAL OF FEEDS	76(76%)
2	RESPIRATORY DISTRESS	57(57%)
3	FEVER	50(50%)
4	LETHARGY	14(14%)
5	ABDOMINAL DISTENSION	11(11%)
6	INCESSANT CRY	10(10%)
7	HYPOTHERMIA	9(9%)
8	CONVULSION	8(8%)
9	VOMITTING	7(7%)
10	DIARRHOEA	3(3%)

Out of 100 neonates, 77% of neonates had refusal of feeds. Many neonates had more than one symptom (36%).

FIGURE-6:CLINICAL FEATURES OF NEONATES WITH SEPTICEMIA(N=100)

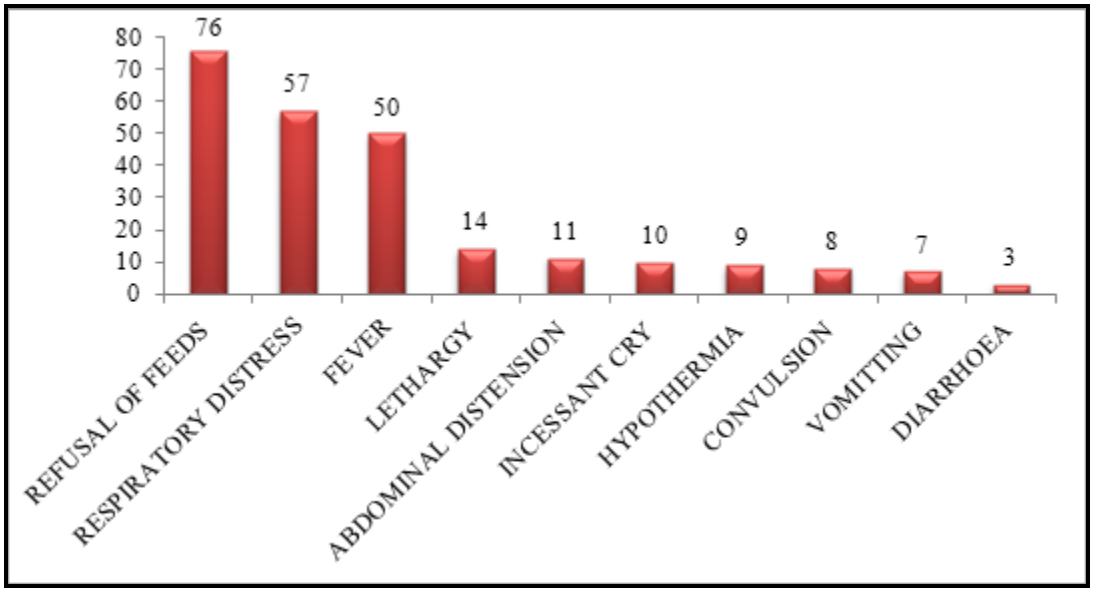
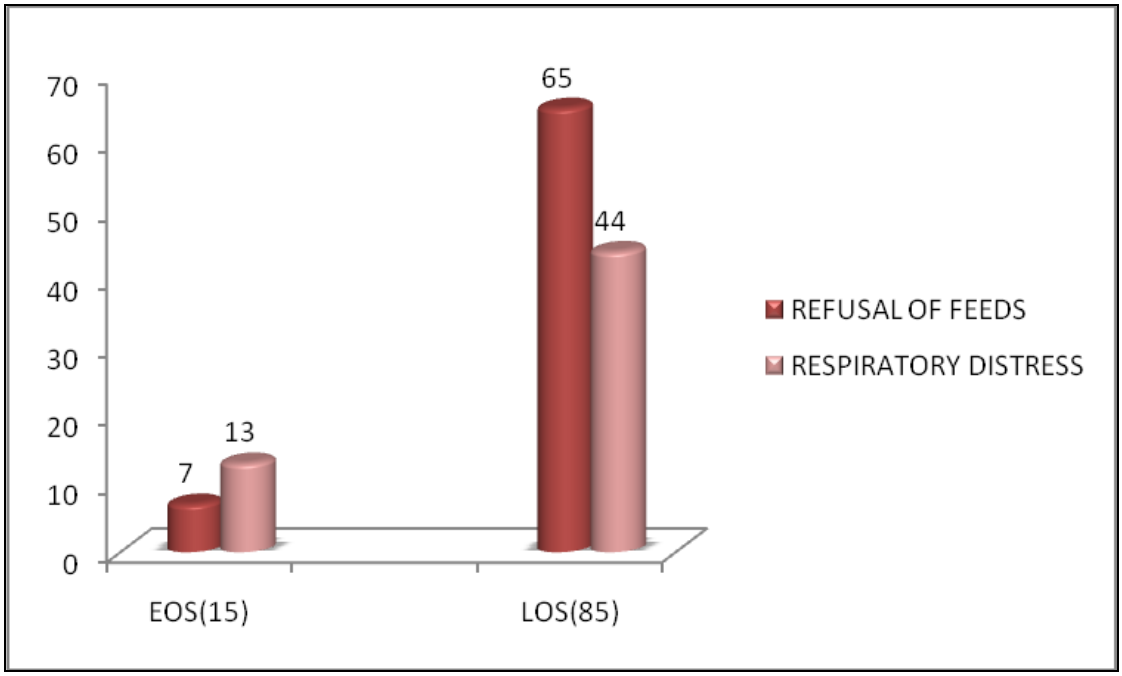


TABLE 7: MOST COMMON CLINICAL FEATURES IN SEPSIS

ONSET	REFUSAL OF FEEDS	RESPIRATORY DISTRESS
EOS(15)	11	13
LOS(85)	65	44

FIGURE 7: MOST COMMON CLINICAL FEATURES IN SEPSIS



Among 15 cases of early onset sepsis, respiratory distress was the most common symptom. Among 85 cases of late onset sepsis ,refusal of feeds was the most common symptom.

TABLE 8 :DISTRIBUTION OF CASES AMONG TWO CATEGORIES

Categories	No of Cases
Proven Sepsis	24
Suspected Sepsis	76

Among 100 cases, 24% was culture proven sepsis.

FIGURE 8 :DISTRIBUTION OF CASES AMONG TWO CATEGORIES

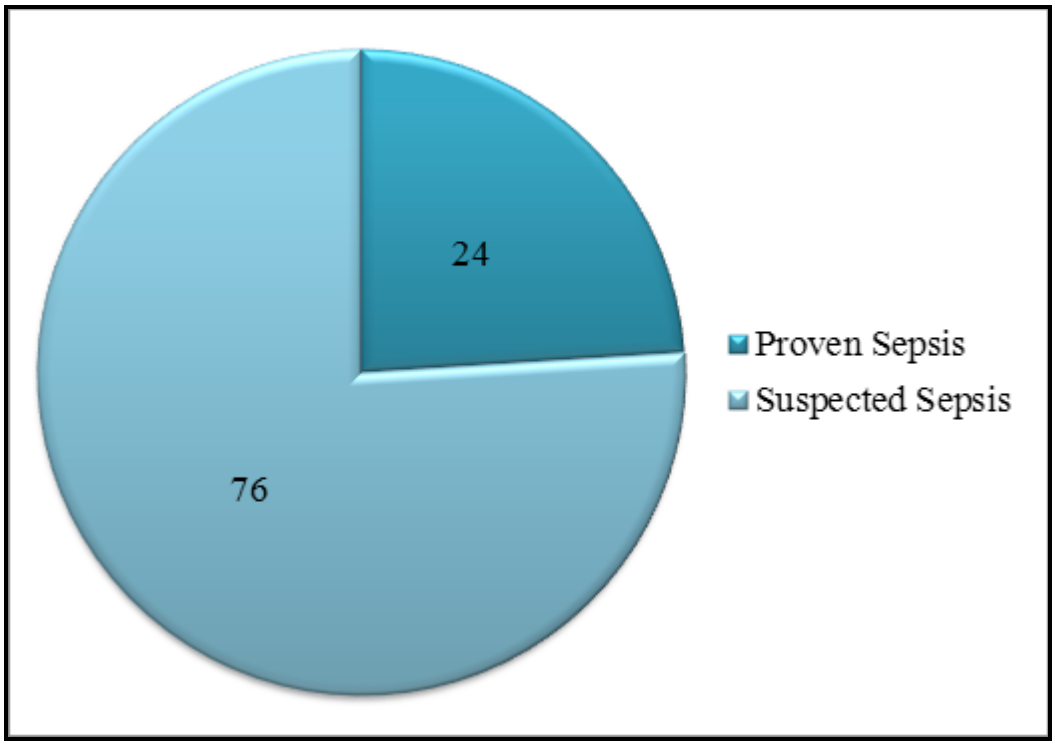


TABLE-9: ANALYSIS OF BACTERIAL ISOLATES IN NEONATAL SEPSIS

Organisms	Frequency	Percentage
GNB	16	59.4
GPC	8	40.6

In neonatal sepsis, 59.4% of sepsis caused by gram negative organisms.

FIGURE-9: ANALYSIS OF BACTERIAL ISOLATES IN NEONATAL SEPSIS

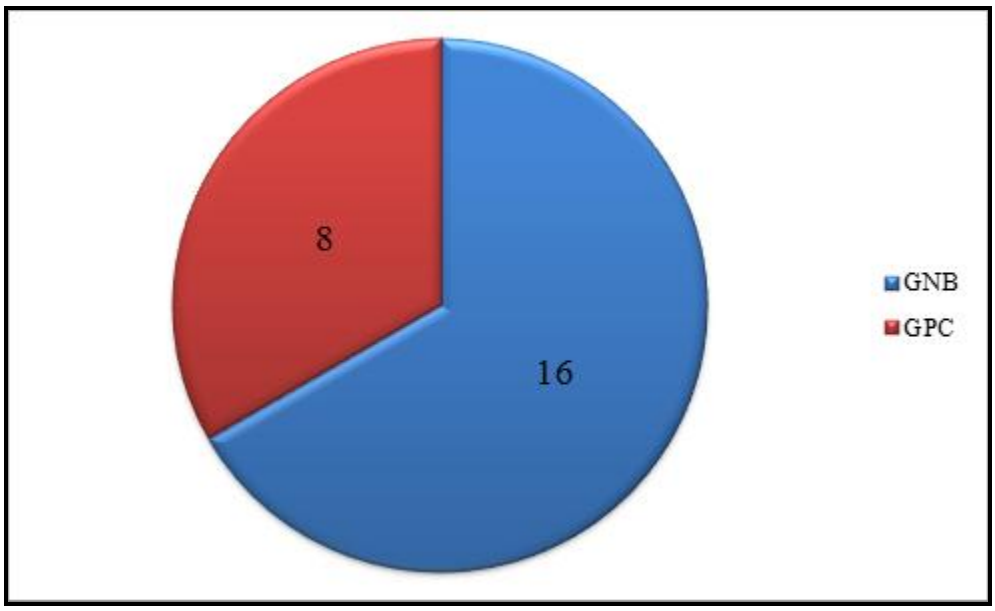


TABLE 10: ORGANISMS WISE DISTRIBUTION IN EOS AND LOS

ORGANISMS		EOS	LOS
Gram Negative(16)	*Klebsiella pneumoniae*	-	3
	Klebsiella pneumoniae(ESBL)	1	2
	Acinetobacter baumannii	1	3
	Pseudomonas aeruginosa	-	3
	Escherichia coli	-	2
	Pseudomonas stutzeri	-	1
Gram Positive(8)	*Staphylococcus aureus*(MSSA)	-	3
	Staphylococcus aureus(MRSA)	-	2
	Staphylococcus epidermidis	-	2
	Enterococcus faecalis	-	1
Total		**2(8.3%)**	**22(91.7%)**

TABLE 11: DISTRIBUTION OF ORGANISMS IN NEONATAL SEPSIS (N=24)

Organisms		Frequency	Percentage
Gram Negative(16)	*Klebsiella pneumoniae*	6	25
	Acinetobacter baumannii	4	16.7
	Pseudomonas aeruginosa	3	12.5
	Escherichia coli	2	8.3
	Pseudomonas stutzeri	1	4.2
Gram Positive(8)	*Staphylococcus aureus*	5	20.8
	Staphylococcus epidermidis	2	8.3
	Enterococcus faecalis	1	4.2
Total		**24**	**100**

It was found that out of 100 samples processed, gram negative bacilli were isolated from 16(66.7%) blood samples.

Among gram negative bacilli, 37.5% were *Klebsiella pneumoniae,* 18.75% were *Pseudomonas aeruginosa, 6.*25% were *Pseudomonas pstutzeri,*25% were *Acinetobacter baumannii* and 12.5% were *Escherichia coli.*

Among gram positive bacilli, 62.5% were *Staphylococcus aureus,* 25% were *Staphylococcus epidermidis* and 12.5% were *Enterrococcus faecalis* **.** Gram negative organisms were more common in neonatal septicaemia. Among gram negative organisms *Klebsiella pneumoniae* and among gram positive organisms *Staphylococcus aureus* were the most common isolates.

FIGURE-10: DISTRIBUTION OF ORGANISMS IN NEONATAL SEPSIS (N=24)

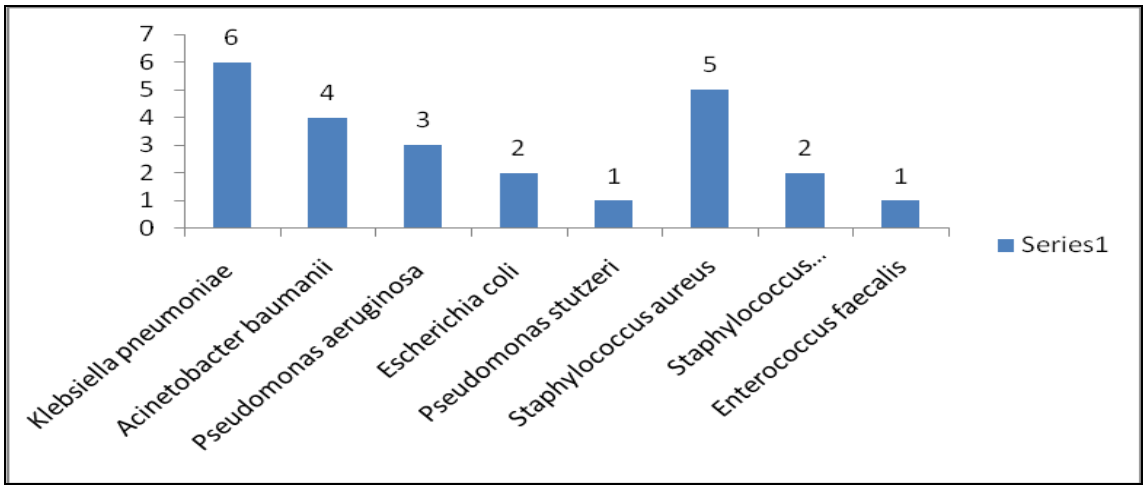


TABLE-12: ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR GRAM NEGATIVE ISOLATES IN NEONATAL SEPSIS CASES (N=16)

Name of Antibiotics	*E coli* (n=2)		*Klebsiella pneumoniae* (n=6)		*Pseudomonas aeruginosa* (n=3)		*Pseudomonas stutzeri* (n=1)		*Acinetobacter baumanii* (n=4)	
	Sen	%	Sen	%	Sen	%	Sen	%	Sen	%
Amikacin(30µg)	2	100	5	83.3	3	100	1	100	2	50
Gentamicin(10µg)	0	0	0	0	3	100	0	0	0	0
Ceftazidime(30µg)	1	50	6	100	3	100	1	100	3	75
Cefotaxime(30µg)	1	50	3	50	0	0	0	0	1	25
Ciprofloxacin(5µg)	1	50	5	83.3	3	100	1	100	1	25
Cotrimoxazole (1.25/23.75µg)	0	0	0	0	-	-	-	-	0	0
Piperacillin/ tazobactum(100/10µg)	2	100	6	100	3	100	1	100	4	100
Imipenem(10µg)	2	100	6	100	3	100	1	100	4	100

In the present study, Out of 100 cases of neonates, 24 were culture positive. Out of this, 16 were gram negative organisms. The GNB's were 87.5% sensitive to amikacin , 81.25% showed sensitive to ceftazidime, 68.75% sensitive to fluroquinolones.

Pseudomonas aeruginosa isolates showed 100% sensitive to aminoglycosides , third generation cephalosporins. *Acinetobacter baumanii* was 50% sensitive to amikacin, 75% sensitive to ceftazadime. All GNB were 100% sensitive to imipenem, pipercillin- tazobactum.

TABLE-13: TOTAL NUMBER OF ESBL PRODUCER IN NEONATAL SEPSIS

Organism	ESBL producer	Non esbl
Klebsiella Pneumoniae(6)	3(50%)	3(50%)

There was no MBL, AMPC detected in the present study.

TABLE-14 : ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR GRAM POSITIVE ISOLATES IN NEONATAL SEPSIS CASES (N=8)

NAME OF ANTIBIOTICS	***Staphlococcus aureus* (n=5)**		***Stapyhlococcus epidermidis* (n=2)**	
	No	%	No	%
Amikacin(30µg)	5	100	2	100
Gentamicin(10µg)	0	0	0	0
Ciprofloxacin(5µg)	5	100	2	100
Cotrimoxazole(1.25/23.75µg)	0	0	0	0
Erythromycin(15µg)	0	0	0	0
Penicillin(10units)	1	20	2	100
Cefoxitin(30µg)	3	60	2	100
Vancomycin (30µg)	5	100	2	100

Out of seven Staphylococcus species, 5 isolates were *Staph aureus* and 2 isolates were *Staphylococcus epidermidis*. All *Staphylococcus* species showed 100% sensitive to vancomycin by minimum inhibitory concentration method(MIC). *Staphylococcus aureus* showed 60% sensitive to methicillin, 20 % were Methicillin resistant *Staphylococcus aureus*(MRSA). All gram positive cocci were 100% sensitive to fluroquinolones. *Enterrococcus faecalis* was isolated in one sample that was 100% sensitive to High level gentamycin(HLG).

TABLE-15 : INTERPRETATION OF MIC OF VANCOMYCIN FOR METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS BY MACROBROTH DILUTION METHOD

Number of MRSA Isolates	MIC value	Interpretation
2	≤2µg/l	Sensitive

INTERPRETATION

≤ 2µg/ml- Susceptible

4-8 µg/ml- Intermediate

≥ 16 µg/ml- Resistant

TABLE-16: ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR *ENTEROCOCCUS FAECALIS* IN NEONATAL SEPSIS CASES (N=1)

Name of Antibiotics	*Enterococcus faecalis* (n=1)	
	No	%
High Level Gentamicin (120µg)	1	100
Ampicillin(10µg)	0	0
Erythromycin(15µg)	0	0
Penicillin(10µg)	0	0
Vancomycin(30µg)	1	100

TABLE-17: SYSTEMIC INFECTIONS ASSOCIATED WITH NEONATAL SEPSIS (N=100)

SYSTEMIC INFECTIONS	NO. OF CASES(%)
BRONCHO PNEUMONIA	14(14%)
MENINGITIS	7(7%)
NNH (DIRECT)	3(3%)
NNEC	2(2%)
SEPTIC ARTHRITIS	2(2%)
SEPTIC ILEUS	1(1%)
PYELONEPHRITIS	1(1%)

NNH= NEONATAL HYPERBILIRUBINEMIA.

NNEC=NEONATAL NECROTIZING ENTEROCOLITIS.

TABLE-18: SERUM PROCALCITONIN LEVEL IN NEONATAL SEPTICEMIA (N=100)

Procalcitonin ng/ml	Number(%)
Negative(< 0.25)	37(37%)
0.25 to 0.5	4(4%)
0.5 to 10	52(52%)
>10	7(7%)

Out of 100 neonates, serum Procalcitonin was elevated in 63% of neonatal sepsis. Among 63 procalcitonin positive cases, Majority of cases seen between 0.5 to 10ng/ml. Procalcitonin level >10 ng/ml indicates severe bacterial sepsis. Less than 0.25ng/ml was considered as PCT negative.

TABLE-19: PROCALCITONIN VS CRP ESTIMATION IN NEONATAL SEPTICEMIA (N=100)

Acute Phase Reactants			Positive	
			Number	Percentage
Procalcitonin			63	63
C-Reactive Protein			43	43
Pearson chi Square test	DF - 1	p- value = 0.004	Significant	

TABLE-20: COMPARISON OF CRP AND PROCALCITONIN IN INFECTION CATEGORIES

	CRP Positive		PCT Positive	
Proven sepsis(24)	15	62.5%	21	87.5%
Suspected sepsis(37)	20	54.05%	27	73%
Clinical sepsis(39)	0	-	15	38.5%

FIGURE-11: COMPARISON OF CRP AND PROCALCITONIN IN INFECTION CATEGORIES

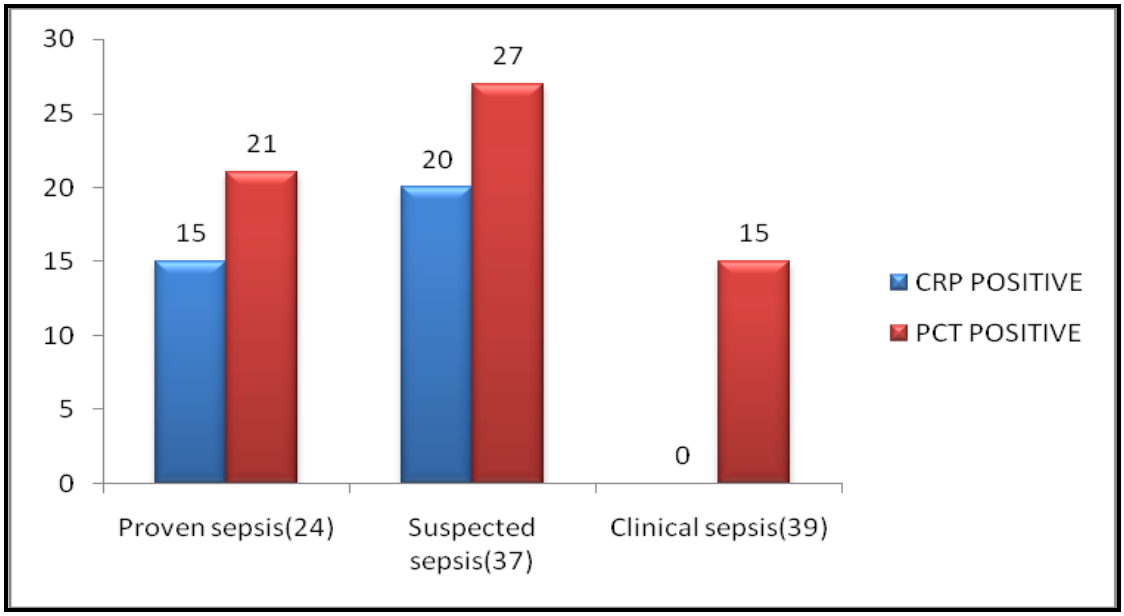


TABLE 19: STATISTICAL ANALYSIS OF PROCALCITONIN

PCT	Blood culture		Total
	Positive	Negative	
POSITIVE	21	42	63
NEGATIVE	3	34	37
Total	24	76	100

Sensitivity=87.5%

Specificity=44.7%

Positive predictive value=33.33%

Negative predictive value=91.8%

TABLE-20: STATISTICAL ANALYSIS OF C-REACTIVE PROTEIN

CRP	Blood culture		Total
	Positive	Negative	
POSITIVE	15	27	42
NEGATIVE	9	49	58
Total	24	76	100

Sensitivity=62.5%

Specificity=65.3%

Positive predictive value=35.7%

Negative predictive value=84.4%

On comparison of serum CRP and Procalcitonin estimation which are indicators of septicemia it was observed that estimation of procalcitonin (63%) detected more cases of septicemia than CRP estimation (43%).

The sensitivity of PCT in detecting sepsis was 87.5% , its specificity was 44.70% , its positive predictive value was 33.33% ,and its negative predictive value was 91.80% . The sensitivity of CRP in detecting sepsis was 62.5% , its specificity was 65.3%, its positive predictive value was 35.7%, and its negative predictive value was 84.4% .

In present study, the total WBC was normal in 11 out of 24 cultures with proven sepsis. In neonates with an elevated PCT level may help in the diagnosis of septicemia . In the present study elevated PCT level were high in both proven and suspected sepsis. In the present study, PCT test detected 87.5% of neonatal sepsis cases than CRP 62.5%. There was a significant correlation between the serum PCT level and type of sepsis (p value=0.004)..

DISCUSSION

This study was conducted in the Institute of Microbiology, Madras Medical College, Rajiv Gandhi Government General Hospital (RGGGH), Chennai in association with Institute of Child Health, Neonatal unit, Egmore.

According to clinical symptoms of sepsis, microbiologic and laboratory results(C Reactive Protein, white cell count, platelet count and blood culture), neonates classified into three groups.

Among three groups, 24 neonates had proven sepsis , in whom 15 neonates had elevated CRP and 21 neonates had elevated PCT. Among 37 neonates with suspected sepsis, 20 neonates had elevated CRP , 27 neonates had elevated PCT. Among 39 neonates with clinical sepsis, elevated CRP not seen but 15 neonates had elevated PCT. The total WBC was elevated in 11 out of 24 culture proven sepsis.

In the present study elevated PCT level seen in both proven and suspected sepsis. This finding was comparable with that of the study which was conducted by **Yodolla Zaheephasia Et al[85] and Monneret et al[86][89].**

In the present study, Among 100 cases of neonatal sepsis, females babies (55%) were predominant group when compared to males (45%). This was contrary to the study conducted by **Roy et al., (2002)** [67]. This predilection of higher frequency among females was attributed towards the presence of risk factors like low birth weight and prematurity [93][95][97-99].

In the present study, Low birth weight babies (63%) were the most common risk factors associated with neonatal sepsis. This was similar to the study conducted by **Stoll et al.,(1996)** [3], **Belady et al., (1997)** [64] **and Kaftan et al., (1998)** [65]**.** The risk factor associated with bacterial sepsis and meningitis was low birth weight according to **Fanaroff et al., (1998)** [66]**.** Reports from India show 50-60% of septic babies were premature babies and very low birth weight babies. This was documented by **Bang et al., (2001)** [2]**.** According to **Roy et al., (2002)** [67] the most frequent neonatal risk factor was low birth weight affecting 63.8% of the neonates.

In this present study, prematurity (41%) was the second most common risk factors associated with neonatal sepsis. This was smilar to study conducted by **Takkar et al., (1974)**[8] **and Singh et al., (1994)** [7] ,**Khatua et al., (1986)** [68]**.**

The increased incidence of septicemia among premature neonates was attributed to their poor immune response like low level of Ig G,

impaired cellular immunity and poor mucosal defence according to **Schreiber et al (1992) [69].**

In the present study, among 59 term babies, Male babies (54.2%) were commonly affected. Neonatal sepsis is predominant among term male infants. Term male infants have an approximately twofold higher incidence of sepsis than term females as stated by **Washburn et al., (1969) [70].** This sex difference is less clear in preterm , low birth weight (LBW) infants . A gene located on the X chromosome is involved with a function of the thymus or with the synthesis of immunoglobulins. The female has double X chromosomes and possess a greater resistance to infection as explained by **Schlegal R J and Bellanli A J (1969) [71].**

In this present study premature rupture of membranes contributed to 10 % of cases of neonatal sepsis. This was similar to the study conducted by **Shah et al (2006) [72]** .

In this present study Late onset sepsis was more common than Early onset sepsis. Late onset sepsis is either hospital-acquired or community acquired and neonates usually present with septicemia, pneumonia or meningitis [13].

In the present study, Refusal of feeds (76%) was one of the important symptoms observed. This was in accordance with study conducted by **Das et al., (1980) [73] and Shashikala et al., (2000) [74]**

were refusal of feeds (61%), respiratory distress (40%), convulsions (29%) and abdominal distension (23%). 50% of infected newborn infants have a temperature higher than 37.8 °C [75]. Feeding intolerance and abdominal distension was 46%; lethargy and hypotonia was 37% as per the report of **Fanaroff et al., (1998)** [66].

In the present study, Among 15 cases of newborns with Early Onset Sepsis, Respiratory distress was the most common presentation. Similar results were obtained in studies conducted by **A.S.M Nowshad Uddin Ahmed et al**[76]. The most frequent clinical presentations of patients with culture proven serious neonatal bacterial infections were respiratory distress (47%), lethargy (40%), fever (36%), poor feeding (27%). Respiratory distress was significantly more common in early than late onset sepsis.

Among 100 cases of neonatal sepsis, 24 were culture proven sepsis, which were correlated significantly (P value=0.004). Gram negative bacilli constituted the predominant isolate (66.7%) followed by the gram positive cocci (33.3%)

The most common organisms among gram negative bacilli was *Klebsiella pneumoniae(25%)* followed by *Pseudomonas aeruginosa*(12.5%), *Pseudomonas pstutzeri(4.2), Acinetobacter baumannii(16.7)* and *Escherichia coli(*8.3%).

In the present study, among gram positive cocci, 20.8% were *Staphylococcus aureus*, 8.3% were *Staphylococcus epidermidis* and 4.2% were *Enterococcus faecalis* **.**

Gram negative organisms were more common in neonatal septicemia. . This was smiilar to the study conducted by **Kurien Anil Kuruvilla et al (1998) [77].** In this study, *E.coli* and *Klebsiella* which were the most common organisms responsible for EOS and LOS respectively. *Enterococcus faecalis* was also found to be a major pathogens in both EOS and LOS. The study on neonatal sepsis by **Trotman H and Bell Y.(2006) [21]** reported 63% of the isolates were gram negative.

ANTMICROBIAL SUSCEPTIBLTY PATTERN:

In the present study, Out of 24 culture positive isolates, 16 gram negative organisms were isolated. Among Gram negatve organisms,87.5% of isolates were sensitive to amikacin , 81.25% showed sensitive to ceftazidime, 68.75% sensitive to fluoroquinolones.

Pseudomonas aeruginosa isolates showed 100% sensitive to aminoglycosides and third generation cephalosporins. *Acinetobacter baumannii* was 50% sensitive to amikacin, 75% sensitive to ceftazadime. All GNB were 100% sensitive to imipenem, piperacillin-tazobactum.

Out of seven *Staphylococcus* species, 5 isolates were *Staphylococcus aureus* and 2 isolates were *Staphylococcus epidermidis*. *Staphylococcus aureus* showed 60% sensitive to methicillin, 20 % were Methicillin resistant *staphylococcus aureus* (MRSA). All *Staphylococcus* species showed 100% sensitivity to vancomycin by minimum inhibitory concentration method(MIC). All gram positive cocci were 100% sensitive to fluroquinolones.

Enterococcus faecalis was isolated in one sample that was sensitive to High level gentamicin (HLG).

In the present study, serum procalcitonin was measured by QDx Instacheck PCT Kit .Among 63 procalcitonin positive cases, 82.5% showed a serum PCT level in the 0.5 to 10ng/ml, 11.1% of cases showed a serum PCT more than 10ng/ml and 6.4% of cases showed a serum PCT level less than 0.5ng/ml. Serum Procalcitonin level >10 ng/ml indicates severe bacterial sepsis. Less than 0.25ng/ml was considered as PCT negative.

This findings was similar to the study conducted by Sucilathangam G.et al, that the serum PCT level was measured by using a quantitative immuno-luminometry method and the Lumitest kit (BRAHMS Diagnostic, Berlin, Germany). In this assay, a PCT level of $\geq$0.5 ng/ml was considered as pathological. PCT levels of 0.5-2 ng/ml,

2-10 ng/ml and >10 ng/ml were considered as weakly positive, positive, and strongly positive, respectively [92] [94].

In the present study, the sensitivity of PCT in detecting sepsis was 87.5%, its specificity was 44.70% , its positive predictive value was 33.33% and its negative predictive value was 91.80%. This finding was comparable with that of the study which was conducted by Chiesa et al [47] (1998)reported that the sensitivity of diagnosing sepsis in neonates by PCT during the first 48 hours of life as 85.7% ; the sensitivity of detecting late onset sepsis was 100%.

Infants receiving antibiotic therapy within the previous 72 hrs of PCT tests could also show decreasing levels of PCT. From the study by Ugarte and colleagues (1998) at a cutoff value of 0.6 ngm / ml., PCT had a sensitivity of 67.6%, a specificity of 61.3%[96].

In this study, the estimation of serum C-Reactive Protein was done by GenX CRP Turbilatex Quantitative method. The sensitivity of CRP in detecting sepsis was 62.5% , its specificity was 65.3%, its positive predictive value was 35.7% and its negative predictive value was 84.4%. The correlation between serum PCT and CRP was compared .Higher proportion of the neonates with sepsis had raised PCT than those without sepsis.

In the present study, sensitivity of serum procalcitonin (87.5%) for detection of neonatal sepsis was higher than the sensitivity of CRP

(62.5%). In most of the culture positive cases, the other sepsis screening tests were negative , but the level of PCT was elevated. This was similar to Boo et al findings. These findings support the usefulness of PCT in establishing an early diagnosis of neonatal sepsis. **Carot et al[11]** in their study showed that procalcitonin is more sensitive than the CRP in the diagnosis of septicemia, meningitis, urinary tract infections[100][102][107].

The present study confirmed the findings of other investigators that PCT was more sensitive than CRP in the detection of neonatal sepsis. In a recent study , **Koksal et al[31]** concluded that serum procalcitonin level was superior to serum CRP level in terms of early diagnosis of neonatal sepsis, in detecting the severity of the illness and in evaluation of the response to antibiotic treatment [97][108][88][101].

SUMMARY

- 100 cases of newborn who fulfilled the inclusion criteria were included in this study

- Females were most commonly affected than males in neonatal sepsis .Among term infants, male babies were commonly affected.

- Late onset sepsis (after 72 hours of life) was more common (85%) than early onset sepsis (15%). Majority of babies were affected during the early neonatal period.

- In the present study, overall 60% of neonates born by normal delivery were also affected.

- The main risk factors involved in neonatal sepsis were low birth weight(63%) and prematurity(41%).

- Refusal of feeds(76%) was the most common symptom of neonatal septicemia.

- The prevalence of neonatal septicemia according to the present study was 24%.

- Out of 24 culture positive isolates, 16 (66.7%) were gram negative Bacilli and 8(33.3%) were gram positive organisms, which was correlated significantly (p value= 0.004).

- *Klebsiella pneumoniae* (25%) was the most common isolate in neonatal sepsis, followed by *Staphylococcus aureus* (20.8%), *Acinetobacter baumannii*(16.7%), *Pseudomonas aeruginosa* (12.5%).

- All GNB's were sensitive(100%) to carbapenem.

- Among of 8 gram positive cocci (*Staphylococcus* species), 6 isolotes were methicillin sensitive (75%) and 2 were Methcillin resistant (25%).The two MRSA solates were found to be sensitive to Vancomycin by macrobroth dilution method.

- In this study, one *Enterococcus faecalis* was isolated that was sensitive to high level gentamicin.

- In the present study, Serum procalcitonin was elevated in 21 out of 24 culture proven sepsis. Serum C-Reactive protein was elevated in 15 out of 24 culture proven sepsis. The total WBC was elevated in 11 out of 24 cultures with proven sepsis. In neonates with an elevated PCT level may help in the diagnosis of septicemia. In the present study elevated PCT level were high in both proven and suspected sepsis.

- The sensitivity of PCT in detecting sepsis was 87.5%, its specificity was 44.70%, its positive predictive value was 33.33% and its negative predictive value was 91.80%.

- The sensitivity of CRP in detecting sepsis was 62.5% , its specificity was 65.3%, its positive predictive value was 35.7% and its negative predictive value

- **S**ince the serum PCT levels were elevated in almost all the culture proven sepsis cases, PCT can be used as a good tool for the diagnosis of neonatal sepsis and for treating the sepsis cases.

- PCT was highly specific for bacterial infections (endotoxins released from the bacterial cell wall) and it helps differentiating it from viral infection.

- It correlates well with the progression and the severity of the infection.

- Evaluation of PCT levels helps in an early diagnosis of the sepsis on the day of admission itself, before the blood culture is ready (usually after 3days of sampling). PCT helps in avoiding unnecessary, increased antibiotic usage where it is not required and thereby reducing the cost and the occurrence of bacterial resistance. PCT can also be employed for the prognosis of sepsis.

- The findings of the present study confirmed that the serum levels of PCT was a reliable marker of sepsis than the serum levels of CRP and the WBC counts in the early diagnosis of neonatal sepsis

and in the evaluation of the response of the disease to the antibiotic therapy.

- The benefits of measuring serum PCT helps in the diagnosis of neonatal sepsis supports the acceptance of the test in the route practice.

- In the present study, the mortality rate due to sepsis related causes was 5%.

CONCLUSION

This study was conducted in the Institute of Microbiology, Madras Medical College, Rajiv Gandhi Government General Hospital (RGGGH), Chennai in association with Institute of Child Health, Neonatal unit, Egmore. The most common organisms was *Klebsiella pneumoniae(25%)* followed by *Staphylococcus aureus*(20.8%).

In the present study, the sensitivity of the procalcitonin (87.5%) was higher than the sensitivity of CRP (62.5%) for the diagnosis of neonatal sepsis and also the early diagnosis of neonatal sepsis and antimicrobial therapy improve the outcome of the patients. Blood culture is a gold standard method to detect the organisms but the result is delayed(usually after 3days of sampling. Hence, initiation of immediate treatment is made possible by early detection of procalcitonin estimation.

The estimation of serum procalcitonin routinely in the diagnosis and follow up of neonatal sepsis, reduces the hospital cost, avoiding unnecessary, increased antibiotic usage where it is not required and the occurrence of bacterial resistance. In conclusion, the findings of the present study confirm that serum levels of PCT was a more reliable marker than the serum levels of CRP and WBC counts in the early diagnosis of neonatal sepsis and in the evaluation of the response of the disease to the antibiotic therapy.

APPENDIX-I

ABBREVIATIONS

ATCC	:	American Type Culture Collections
CLSI	:	Clinical and Laboratory Standards Institute
CONS	:	Coagulase negative Staphylococcus.
CRP	:	C-Reactive Protein
DIC	:	Disseminated Intravascular Coagulation
EOS	:	Early Onset Sepsis
ESBL	:	Extended spectrum beta-lactamase
GNB	:	Gram negative Bacilli
GPC	:	Gram positive Cocci
LOS	:	Late Onset sepsis
MIC	:	Minimum Inhibitory Concentration
MRSA	:	Methicillin-resistant Staphylococcus aureus
MSSA	:	Methicillin-susceptible Staphylococcus aureus
NICU	:	Neonatal intensive care unit
PCT	:	Procalcitonin

FIGURE 1:GROWTH OF STAPHYLOCOCCUS AUREUS ON MACCONKEY AGAR

FIGURE 2:GROWTH OF KLEBSIELLA PNEUMONIAE ON MACCONKEY AGAR

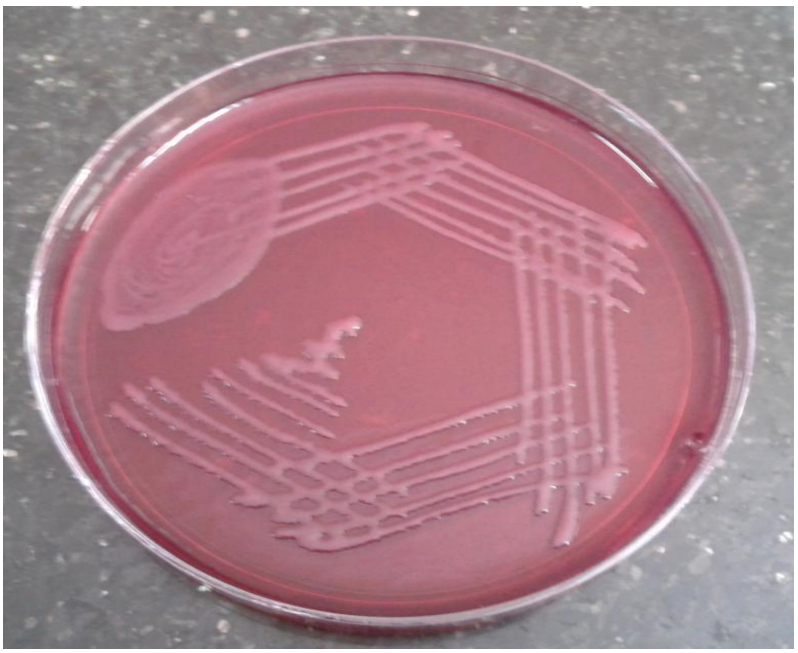


FIGURE 3 :BIOCHEMICAL REACTIONS OF KLEBSIELLA PNEUMONIAE

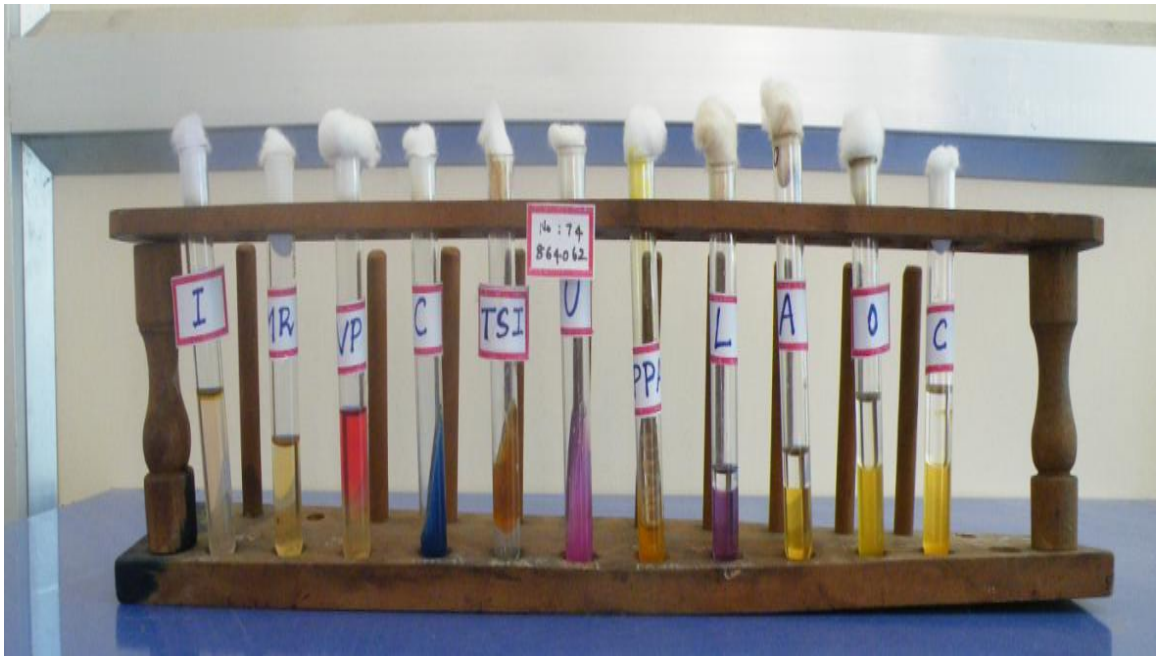
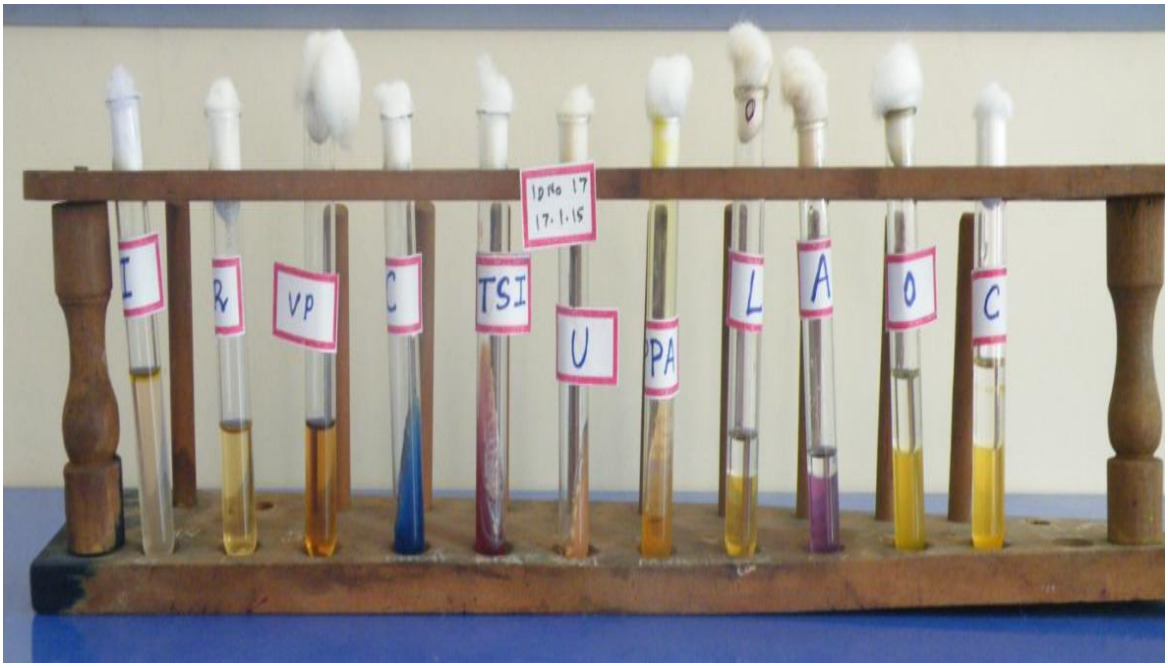


FIGURE 4 :BIOCHEMICAL REACTIONS OF ESCHERICHIA COLI



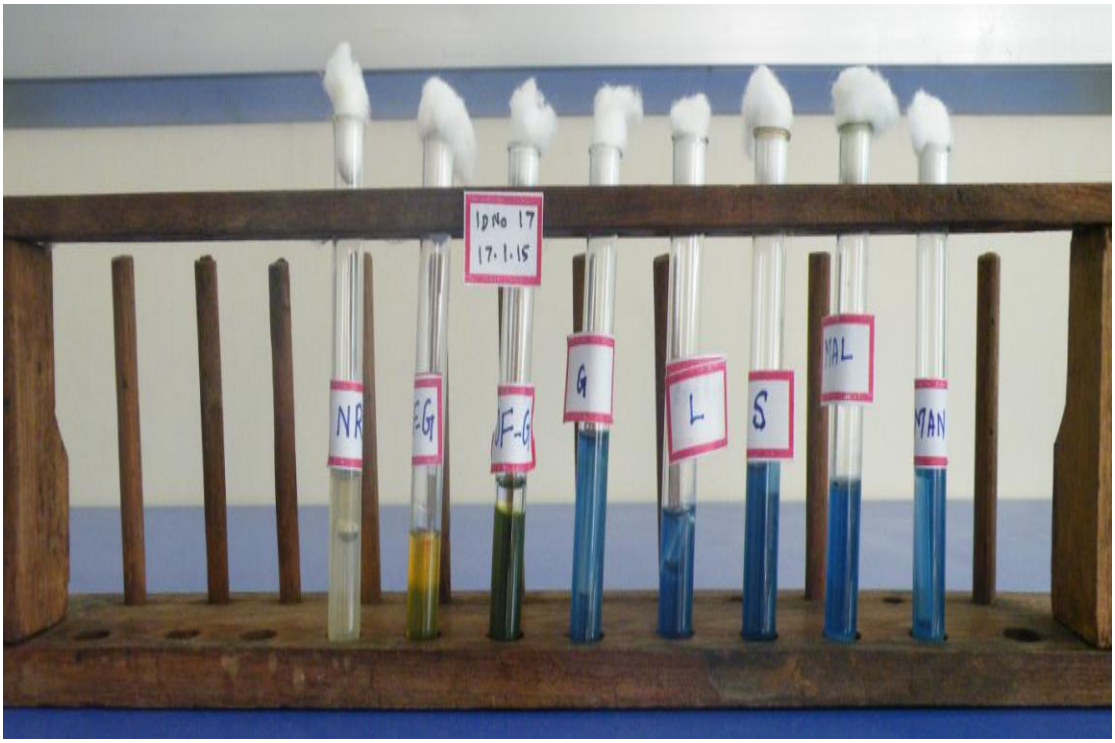


FIGURE 5 :BIOCHEMICAL REACTIONS OF ENTERROCOCCUS FAECALIS

FIGURE 6:ANTIBIOGRAM OF MRSA SHOWING CEFOXITIN RESISTANCE

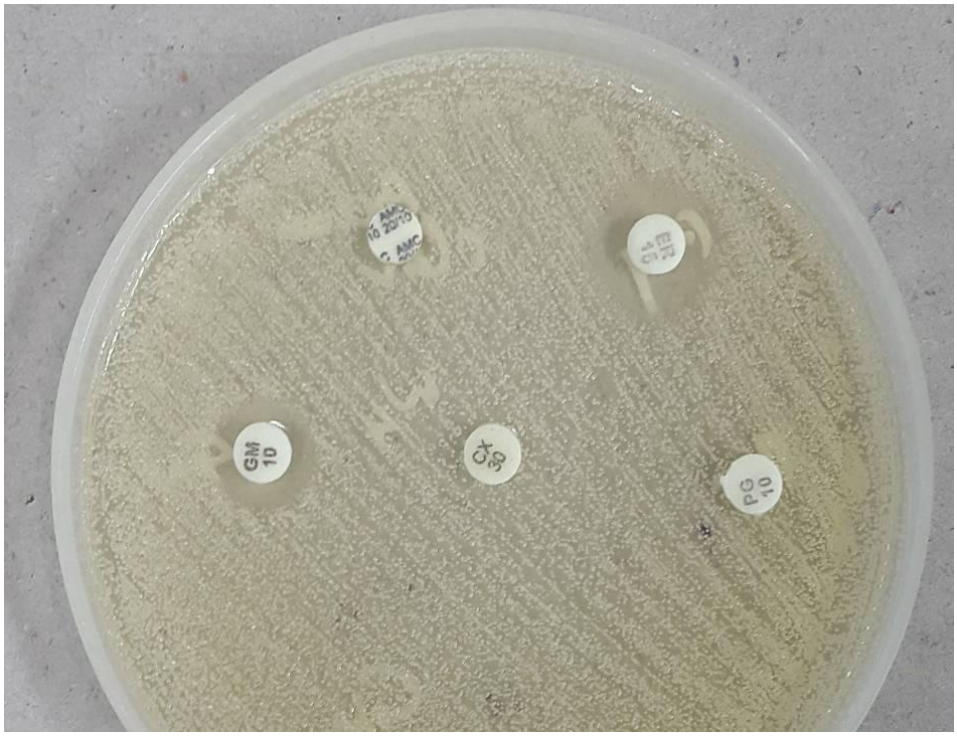


FIGURE 7:PHENOTYPIC CONFIRMATION DISC DIFFUSION TEST (PCDDT) FOR ESBL PRODUCTION

FIGURE 8: DETERMINATION OF VANCOMYCIN MIC FOR MRSA ISOLATES BY MACROBROTH DILUTION METHOD

FIGURE 9: VANCOMYCIN MIC BY E-TEST FOR MRSA

FIGURE 10: QDx Instacheck ™ PCT CARTRIDGE WITH DETECTION BUFFER

FIGURE 11 : THE MEASUREMENT OF PROCALCITONIN QDx Instacheck™ Reader

FIGURE 12: QUANTITATIVE GenX CRP Turbilatex Kit

FIGURE 14: QUANTITATIVE CRP MEASUREMENT BY SEMI-AUTOANALYZER

APPENDIX II

A. STAINS AND REAGENTS

1. Gram staining	l0g Methyl violet in 100ml absolute
Methyl violet (2%)	alcohol in 1 litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolourising agent
Carbol fuchsin 1%	Secondary stain.

B. MEDIA USED

1. Mac Conkey agar

Peptone	20g
Sodium taurocholate	5 g
Distilled Water	1 ltr
Agar	20 g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	l00ml

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix.Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

2. Nutrient agar

Peptic digest of 5g

animal tissue

Sodium chloride	5g
Beef extract	1.5g
Yeast extract	1.5g
Agar	15gm

Final pH 7.4±0.2

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (120°C) for 15 minutes.

3. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	l0g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

4. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution.

5. **Cation adjusted Mueller- Hinton Agar**

Beef infusion	300ml
Caesein hydrolysate	17.5g
Starch	1.5g
Agar	l0g
Distilled water	lltr

pH = 7.4

Sterilise by autoclaving at 121°C for 20 minutes

C. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1. Oxidase Reagent

Tetra methyl p-phenylene diamine dihyrochloride- 1% aqueous solution.

2. Catalase

3% hydrogen peroxide

3.Coagulase test:-

Human plasma was used for performing the coagulase test. This was obtained by centrifuging human blood, with added 0.1% EDTA, at 2000 rpm for 10 minutes.

A.Slide coagulase test:-

One or two staphylococcal colonies were emulsified in a drop of saline on a clean microscopic slide. If the strain was not autoagglutinable,

then undiluted plasma was added to the suspension using a Pasteur pipette. The appearance of coarse clumping visible to the naked eye within 5-10 seconds was taken as positive. Positive and negative controls were put up, to check the proper reactivity of plasma. Absence of clumping or any reaction taking more than 10 secondss were taken as negative slide coagulase test.

B.Tube coagulase test:-

1 in 6 dilution of the plasma was prepared in normal saline (0.85% NaCl) and 1 ml volume of it was distributed in small tubes. One - two staphylococcal colonies were inoculated and emulsified in the diluted plasma. Positive control and negative control was put up using *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* respectively. To rule out spontaneous clotting of plasma, a tube of uninoculated plasma was taken. The tubes were incubated at 37°C for upto 4 hours and were observed at 1 hour, 2 hours and 4 hours by tilting the tubes through 90°. The tubes which showed any degree of clot formation were taken as positive. The tubes in which the plasma remained wholly liquid or showed flocculent or ropy precipitate were read as negative. The negative tubes were left at room temperature overnight and re-examined in the next day.

4. Indole test

Kovac's reagent

Amyl or isoamyl alcohol 150ml Para dimethyl amino benzaldehyde lOg Concentrated hydrochloric acid 50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

5.Christensen's Urease test medium

Peptone	lg
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	10ml
Sterile 20% urea solution	100ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

6. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20 g
Bromothymol blue	0.2% 40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

7. Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	lg
Lactose	10 g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
Sodium thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	12ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

8. Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red	l0mg
Ethyl alcohol	30ml
Distilled water	20ml

Voges Proskauer Reagent

Reagent A: Alpha naphthol	5g
Ethyl alcohol	100ml
Reagent B: Potassium hydroxide	40g
Distilled water	100ml

8. Peptone water fermentation test medium

To the basal medium of peptone water, add sterilised sugars of 1% indicator bromothymol blue with Durham's tube. Basal medium peptone water Sugar solutions:

Sugar	1ml
Dislilled water	100ml

pH = 7.6.

9. Mannitol motility medium

Agar	5g
Peptone	lg
Potassium nitrate	1g
Mannitol	2g

Phenol red indicator

Distilled water 1000ml

pH 7.2

10. Phenolphthalein diphosphate agar

- ✓ Sterilize a 1% aqueous solution of sodium phenolphthalein diphosphate by filtration and store at 4°C
- ✓ Add 10ml of this solution to 1000ml melted nutrient agar cooled to 50°C and pour plates
- ✓ Grow the staphylococcus overnight at 37°C on the medium
- ✓ Invert the plate and pour a few drops of ammonia solution SG 0.88 into the lid
- ✓ Read as positive a culture whose colonies turn bright pink within a few minutes. The colour soon fades.

11. Potassium nitrate broth

Potassium nitrate (KNO3)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclaved.

12. Phenyl alanine deaminase test

Yeast Extract	3g
Dl-Phenylalamine	2g
Disodium hydrogen phosphate	lg
Sodium Chloride	5g
Agar	12g
Distilled water	1 lr
PH	7.4

Distributed in tubes and sterilized by autoclaving at 121° C for 1 5 minutes, allowed to solidify as long slopes.

13. Sugar fermentation medium

Peptone	15g
Andrade's indicator	10 ml
Sugar to be tested	20g
Water	1 litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1M sodium hydroxide has been added to turn the colour of the solution yellow.

Dissolve the peptone and Andrade's indicator in 1 litre of water and add 20g of the sugar; sugars to be tested generally include glucose, sucrose, lactose and maltose. Distribute 3ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by steaming at 100 degree C for 30 min on 3 consecutive days.

ANNEXURE II

PROFORMA

- Name : IP no:
- Age: Ward:
- Sex:
- Occupation:
- Address:
- Birth weight: LBW/ Normal
- Apgar Score:
- Maturity: Preterm / Term / Post Term
- Place of delivery : Tertiary care Hospital / Others
- Blood grouping / Rh typing :
- Duration of labour :
- PROM 24 hrs: Less than 24 hrs / More than
- Associated illness specify : Congenital anomalies if any
- Immunization of Mother with TT: Yes / No
- Antenatal period in Mother:Normal / Abnormal
- H/O maternal fever during last Trimester / Labour : Yes / No
- Previous Obstetrical History :
- H / O Abortions :
- Nature of Specimen :
- Investigation required :
- Date of collection of specimen:
- Results declared : Blood culture/CRP/PCT
- Organisms Isolated :

Hematological investigations:

- TC
- DC
- Hb estimation
- ESR
- Peripheral smear study.

Microbiological investigation:

Sample collected:

- Blood

Direct examination:

Gram’stain:

Blood culture:

- Inoculated intoBHI broth with subculture onto
  - MAC
  - BAP
  - CAP

Isolate identified in blood sample:

Antibacterial susceptibility pattern:

ANNEXURE III

CONSENT FORM

STUDY TITLE:"Early diagnostic markers for neonatal sepsis: comparing procalcitonin and C-Reactive protein with the gold standard culture method "

I……, hereby give consent to participate in the study conducted by Dr.R.Kesavan, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my Baby's Blood sample for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal

Signature/ Thumb impression of the patient/ relative Place:

Patient Name & Address: Date:

Signature of the investigator: Signature of the guide:

INFORMATION SHEET

TITLE

"Early diagnostic markers for neonatal sepsis: comparing procalcitonin and C-Reactive protein with the gold standard culture method "

INVESTIGATOR: Dr.R.Kesavan,
Post Graduate,
Institute of Microbiology,
Madras Medical College,
Chennai - 600003.

GUIDE: Dr. S.Thasneem Banu, MD.,
Professor of Microbiology,
Institute of Microbiology,
Madras Medical College,
Chennai 600 003.

Early recognition and diagnosis of neonatal sepsis are difficult because of nonspecific clinical presentation.It is important to make an early diagnosis of neonatal sepsis for the prompt institution of antimicrobial therapy which improves outcome.

Although isolation of the causative microorganisms by using blood culture has been the golden standard method for its diagnosis, the result is ready only 24-72 hrs after the sampling and during this period, it is necessary to treat the suspicious infants for sepsis with antibiotics on the basis of the clinical symptoms and the risk factors.

The present trend which is being applied for infants who are suspected to have neonatal sepsis may lead to unnecessary and increased antibiotic consumption, a higher incidence of the side effects due to their use, increased resistance to the antibiotics, a long hospitalization, the separation of the infants from their mothers and increased health costs. Therefore, using fast diagnostic methods including laboratory markers could be beneficial for the diagnosis of neonatal sepsis .

I am doing to collect blood samples from clinically suspected cases of newborns are included in this study after getting informed consent only. This study is entirely voluntary and patient can withdraw any time from this study. Extra cost will not be incurred to the patients in this study. Any doubt regarding this study will be willingly clarified. Results of the study will be published. In case of any doubt please contact Dr. R.Kesavan, Cell: 9597194456

Signature/ Thumb impression of the patient/ relative Place:

Patient Name & Address: Date:

Signature of the investigator: Signature of the guide:

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 011 25363970

CERTIFICATE OF APPROVAL

To
Dr. R.Kesavan
Postgraduate M.D.(Microbiology)
Madras Medical College
Chennai – 600 003.

Dear Dr. R.Kesavan,

The Institutional Ethics Committee has considered your request and approved your study titled **"Early diagnostic markers for neonatal sepsis : comparing procalcitonin and C-Reactive protein with the gold standard culture method". No.22112014.**

The following members of Ethics Committee were present in the meeting held on 11.11.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandini, M.D., Inst.of Pharmacology, MMC	: Member
5. Prof.P.Ragumani, M.S., Professor, Inst.of Surgery, MMC	: Member
6. Prof.Md.Ali, M.D., D.M., Prof. & HOD of Medl.G.E., MMC	: Member
7. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
8. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
9. Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
10. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
11. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
12. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

22/11/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

BIBLIOGRAPHY

1) Bailey and Scott, “Diagnostic Microbiology” Mosby Elsevier 2012 XIII edition page No. 787-792.

2) Bang AT, Bang RA, Bactule SB, Reddy HM, Deshmukh MD. Effect of home-based neonatal care and management of sepsis on neonatal mortality: field trial in rural India. Lancet 1999;354:1955-61.

3) Stoll BJ. The global impact of neonatal infection. Clin Perinatol 1997;24:1-21 .

4) Report of the National Neonatal Perinatal Database (National Neonatology Forum) 2002-03.

5) Goldman S, Ellis R, Dhar V, Cairo MS. Rationale and potential use of cytokines in the prevention and treatment of neonatal sepsis. Clin Perinatol 1998;25:699-710 .

6) Polinski C. The value of white blood cell count and differential in the prediction of neonatal sepsis. Neonatal Netw 1996;15:13-23 .

7) Singh M, Narang A, Bhakoo ON. Predictive perinatal score in the diagnosis of neonatal sepsis. J Trop Pediatr.1994 Dec;40(6):365-8

8) Takkar VP, Bhakoo ON, Narang A. Scoring system for the prediction of early neonatal infections. Indian Pediatr. 1974;11:597-600 .

9) Baltimore RS. Neonatal nosocomial infections. Semin Perinatol 1998;22:25-32 .

10) Da Silva O, Ohlsson A, Kenyon C. Accuracy of leukocyte indices and C-reactive protein for diagnosis of neonatal sepsis: a critical review. Pediatr Infect Dis J 1995;14:362-6 .

11) Carol ED, Thomason AP, Hart CA. Procalcitonin as a marker of sepsis, Int J Antimicrob Agents 2002;20:1-9

12) Haque KH,Infection and Immunity in the Newborn.In Forfar and Arneil's textbook of Pediatrics 5th edition, 1988; pg 273- 289.

13) Wolach B. Neonatal sepsis: pathogenesis and supportive therapy. Semin Perinatol1997;21:28-38 .

14) Gerdes JS, Polin R. Early diagnosis and treatment of neonatal sepsis. Indian J Pediatr 1998;65:63-78.

15) Moreno MT,Vargas S,Poveda R , et al. Neonatal sepsis and meningitis in a developing Latin American country. Pediatr Infect Dis J 1994;13:516-20

16) Robilland PY , Nabeth P, Hulsey TC et al. Neonatal bacterial septicemia in a tropical area. First year experience in Guadeloupe (French West Indies). Acta Pediatr 1993;82:687-9.

17) Eriksson M, Bennett R.Faecal bacterial micro flora of new born infants during intensive care management and treatment with five antibiotic regiments. Pediatric Infectious Disease Journal 5:533-539.

18) Eriksson M, Melen B. Bacterial colonization of newborn infants in a neonatal intensive care unit. Acta Pediatrics Scandinavia 71:779-783.

19) Bialkowska Hobrzanska H. Molecular characterization coagulase negative staphylococcal surface flkra of premature neonates. Journal of General Microbiology vol 139;p- 2939-2944.

20) Janet M Rennie Textbook of Neonatology, Third edition , P 1109-1162.

21) Trotman H and Bell Y. Neonatal sepsis in very low birth weight infants at the University Hospital of the West Indies. West Indies Med J 2006;55(3):165.

22) William E. Benitz. Serial Serum C-Reactive protein Levels in the Diagnosis of Neonatal Infection. Pediatrics .Vol . 102 No. 4 October 1998,p.e41.

23) Kalid N.Haque . Pattern of culture proven neonatal sepsis in a district general hospital in United Kingdom. Infect control Hosp Epidemiol 2004 ;25:759-764.

24) Moro M.L. Risk factors for Nosocomial sepsis in newborn intensive and intermediate care units. European journal of pediatrics 1996, Vol. 155 , pp 315-322.

25) Vidya UV Bhave SA. Parenteral nutrition in the management of very low birth weight babies- a randomized controlled trial. Indian Paediatric 1995 Feb 32:165:70

26) Charles Natanson. Selected treatment strategies for deposit shock based on proposed mechanisms of pathogenesis. Annals of Internal Medicine. Vol 120.no.9 p : 771-783.

27) Analia L.Laciar; Raul P. Hasuoka. Symptomic hydrocephalus in a newborn infected with listeria monocytogenes. Brazilian Journal of Microbiology 2000,31:09-11.

28) Olbeg Y, Desinor. Neonatal sepsis and meningitis in Halti. Journal of tropical pediatrics 2004,50(1(:48-50.

29) Christi GG,Shenoy V, Matthai J, Shvananda PG, Venkatesh A. Acinetobacter species in neonates. Indian J Pediatr 1993;vol-30:p-1413-1416.

30) Joshi SG , Neonatal grab negative bacteremia. Indian J pediatr 2000 Jan 67:27-32.

31) Koksal N, Hacimustafaiglu M, Bagci S,Celebi S(2001). Meropenem in Neonatal severe infections due to multiresistant GNB . Indian J Paediatr, 68(1):15-9

32) Roilides E, kyriakifdes G, Kadiltsoglou I, Farmaki E, Venzon D, Katsavali A, Kremeno Poulos G (2000). Septicemia due to multiresistant klebsiella pneumoniae in a neonatal unit: a control study. American Journal Of Perinatology,17(1);35-9.

33) G J McGarrity and L L Coriell. Epidemiology of Pseudomonas aeruginosa infections: determination by pyocin typing.Journal of Clinical Microbiology,(1976),vol 3;p264-271.

34) William Taeush H . Avery's disease of newborn 8 th edition. P 551-568.

35) McCracken et al; relationship between E.coli K1 capsular polysaccharide antigen and clinical outcome neonatal meningitis. Lancet 1974;2:246.

36) Idhu Shukla,Solmaz Siddiqui, Sed manazir Ali: Acinetobacter sepsis in neonates, Indian J. Pathol Microbiology Vol.49,pg 59,2006.

37) Vinodkumar CS.Acinetobacter septicemia in neonates. Indian Journal of Medical Microbiology,(2004)22(1):71.

38) Phillip on A, Labia R and Jacoby GA. Extended spectrum beta lactamases. Antimicrobial affects Chemotherapy 1989;33:1131-1136.

39) Shanmuganathan C. Learning from an outbreak : Indian Journal of Medical Microbiology, (2004)22(4):255-257.

40) Jacoby GA . antimicrobial resistant pathogens in the 1990s. Annu Rev. Med 1996; 47. 169-179

41) Jain A, Roy I, Gupta MK, Kumar M and Agarwal Sk. prevalence of ESBL producing gram negative bacteria in septicemic neonates in a tertiary care hospital. Journal of medical Microbiology 2003;52:421-425.

42) Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. I.Refernce values for neutrophilic cells. J Pediatr 1979;95:89-98 .

43) Mouzinho A, Rosenfeld CR, Sanchez PJ, Risser R. Revised reference ranges for circulating neutrophils in very-low-birth-weight neonates. Pediatrics 1994;94:76-82.

44) Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. Hospital-acquired neonatal infections in developing countries. Lancet 2005;365:1175-88.

45) Gerder JS, Polin R, Early diagnosis and treatment of neonatal sepsis. Indian J. Pediatrics 1970; 46, 849-854.

46) Sadana S, Mathur NB, Thakur A. Exchange transfusion in septic neonates with sclerema: effect on immunoglobulin and complement levels. Indian Pediatr 1997;34:20-5 .

47) Chiesa C,Panero A, Rossi N et al. Reliability of Procalcitonin concentration for the diagnosis of sepsis in critically ill neonates. Clin Infect Dis 1998; 26 L 664- 672.

48) Resch B, Gusenleitner W, Huller WD, Procalcitonin and Interleukin-6 in the diagnosis of early onset sepsis of the neonate. Acta pediatrica, 2003; 92: 243-245.

49) Carrigans S D, Scolt G, Tabrizian M, Toward resolving the challenges of sepsis diagnosis. Clin .Chem. 2004: 50: 1301- 1314.

50) Simon L, Gauvin F, Amre DK et al., Serum procalcitonin and C Reactive protein levels as markers of bacterial infections. A systematic review and metaanalysis. Clin Infect Dis 2004; 39:206-217.

51) Shine B, Gould J , Campbell C, Serum C- Reactive protein in normal and infected neonates. Clin Chim Acta 1985; 148:97-103.

52) Wagle S, Granaug A, Kohan R, Evans S, C-Reactive protein as a diagnostic tool of sepsis in very immature babies. J. Pediatr Child Health 1994; 30:40-44.

53) Khassawneh M, Hayajnehwa, Kofahi H et al: Diagnostic markers for neonatal sepsis; Comparing C-Reactive Protein, Interleukin – 6 and Immnunoglobulin M. Scand J Immunol. 2007; Feb; 65 (2); 171-5.

54) Philip A.G.S and Hewitt JR. Early diagnosis of neonatal sepsis. Pediatrics 1980;65; 1036.

55) Murex diagnostics, Murex CRP, N6rcross GA: Inc :1995.

56) Jaye D, Waites K. Clinical applications of C-reactive protein in Pediatrics. Pediatr Infect Dis J 1997; 16: 735-746.

57) Gladstone IM,Ehrenkranz RA, Edberg SC et al., A ten year review of neonatal sepsis and comparison with the previous fifty years experience.pediatr Infect Dis 1990;J 9: 819.

58) Upadhyay A, Aggarwal R, Kapil A, Singh S, Paul VK, Deorari AK. Profile of neonatal sepsis in a tertiary care neonatal unit from

India: A retrospective study. Journal of Neonatology 2006;20:50-57.

59) Deorari Ashok K. For the Investigators of the National Neonatal Perinatal Database (NNPD). Changing pattern of bacteriologic profile in Neonatal Sepsis among intramural babies. Journal of Neonatology 2006;20:8-15.

60) Meharbansingh 'care of the newborn' sagar publication 5th edition 1999; page no :215,217,218.

61) Monica Cheesbrough , district laboratory practice in tropical countries, Cambridge university press reprint 2007; page 127.

62) Mackie and Mcartney: Practical Medical Microbiology 14th edition.p 95-124.

63) Clinical Laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing; seventh informational supplement. CLSI document M100-S24. Clinical and Laboratory Standards Institute , Pennsylvania , USA . 2014.

64) Belady PH, Farkouh LJ, Gibbs RS, Intra amniotic infections and premature rupture of membranes Clin Perinatol 1997; Mar, 24 (I) : 1143-1147.

65) Kaftan H, Kinney JS, Early onset neonatal bacterial infections Semin perinatal 1998; Feb :22(1): 15-24.

66) Fanaroff A A, Korones S B, Wright L L et al., Incidence , presenting features, risk factors and significance of late onset septicaemia in very low birth weight infants. Pediatr infect Dis 1998; 17: 594-598.

67) Roy I Jain A Kumar M, Agarwal SK. Bacteriology of neonatal septicemia in a tertiary care hospital of northern India.Indian J. Med.Microbial 2002;20-156-9.

68) Khatua SP,AK,Chatterjee BD , Ghose B, Saha A, Neonatal septicemia, Indian J. Pediatr 1986;53: 504-509

69) Schreiber JR,Berger M. Intravenous immunoglobulin therapy for sepsis in premature babies. J.Pediatr 1992;121:401-404.

70) Washburn TC, Medearis DN Jr. Child B.Sex differences in susceptibility to infections.Pediatrics 1965; 35:57.

71) Schelgel RJ, Bellanli AJ, Increased susceptibility of males to infection. Lancet 1967;2:826.

72) Shah GS, Budhathoki S,Das B K , Mandal RN. Risk factors in early onset neonatal sepsis, Kathmandu Medical Journal 2006; vol 4: no. 2, Issue 14, 187-191.

73) Das PK, Basu K, Chakraborty P, Bhowmilk PK. Clinical and bacteriological infection in metropolitan city based medical college nursery, J. Indian Med Ass, 2010.

74) Shasikala ST, Kasturi AV, Shoba D, Krishna BVS. Clinical bacteriological specimen in Hubli, Indian J Paediatr 2000;169-174.

75) Nelson ' text book of pediatrics' page no 529.15th edition , 1996; page no 798,18 th edition.

76) Ahmed A S M U, Chowdury M A K A, Hoque M, Damstadt GL. Clinical and Bacteriological profile of neonatal septicemia in tertiary level pediatric hospital in Bangladesh. Ind. Pediatr.2002;39:1034-9.

77) Kurien Anil Kuruvilla, Swati Pillai, Mary Jesudasan, Atanu Kumar. Bacterial profile of sepsis in a neonatal unit in south India. Indian Paediatrics, 1998; 35:851-858.

78) Forbes BA,Sahm DF,Weissfeld AS. Bailey and Scott’s Diagnostic Microbiology.12th ed.Missouri:Mosby Elsevier:2007.p.187-213.

79) Baird D Staphylococcus:Cluster forming Gram positive cocci. In:Collee JG,Fraser AG,Marimion BP,Simmons A,edi tors.Mackie and McCartney Practical Medical Microbiology.14th ed.Delhi:Churchill Livingstone Elsevier:2008.p.413-425.

80) Washington WJ,Allen S,Janda W,Koneman E,Procop G,Schreckenberger PWoods G.Koneman's color Atlas and Text Book of Diagnostic Microbiology.6th ed.Baltimore: Lippincott Williams and Wilkins:2006.p.946-1014.

81) Performance standards for antimicrobial susceptibility testing; 21st Informational supplement (M100 – S21) Supplementary table 2A – s1. (2011) Clinical and laboratory standards Institute, Wayne, pa, USA, Clinical and laboratory standards institute.

82) Chaudhary U, Aggarwal R. Extended spectrum blactamases – An emerging threat to clinical therapeutics. Indian Journal of Medical Microbiology 2004; 22 (2): 75 – 80.

83) Clinical and Laboratory Standards Institute. Performance standards for Antimicrobial susceptibility testing; Twenty first informational supplement. CLSI document M100-S21.vol.31 No.1.M02 – A10. CLSI, Wayne. Pennsylvania USA,2011.

84) Screening Tests for _-Lactamase Production, Oxacillin Resistance, mecAMediated Oxacillin Resistance Using Cefoxitin,MIC _ 8 μg/mL, Inducible Clindamycin Resistance, and High-Level Mupirocin Resistance in the Staphylococcus aureus Group. CLSI document M100-S21. Table 2C-S4. Jan 2011.Vol 31.No.1.

85) Zahedpasha Y, Ahmadpour Kachol M, Hajiahmadi M, Haghshenas M. Procalcitonin as a marker o3 sepsis.Iran J Pediatr 2009;19:117-122.

86) Monneret G,Labaune JM,Isaac C,Bienvenu F,Putet G,Bienvenu J. Procalcitonin and C-Reactive protein levels in neonatal infections. Acta Pediatric 1997;86:209-212.

87) Narang A, Rao . R, O.N.Bhakoo. neonatal necrotizing enterocolitis- an epidemiological study. Indian pediatrics. Vol 30, Oct 1993. P-1207-1214.

88) Ballot DE, Perovic O, Galpin J, Cooper PA. Serum procalcitonin as an early marker of neonatal sepsis. *S. Afr Med J* 2004;94:851-54.

89) Basu S, Guruprasad, Narang A, Garewal G. The diagnosis of sepsis in high risk neonates by using a hematologic scoring system. *Indian J Hematolo Blood Transfusion* 1999;17:32-34.

90) Baumgart S, Hall S E, Campos J M, et al. Sepsis with coagulase negative staphylococci in critically ill in newborns. AmJ Dischild 1983; 137;461.

91) Bizzarro MJ, Raskind C, Baltimore RS, Gallagher PG. Seventy five years of neonatal sepsis at Yale: 1928-2003. *Paediatrics* 2005;116: 595-602.

92) Blommendahl J, Janas M, Laine S, Miettinen A, Ashorn P. Comparison of procalcitonin with CRP and the differential white blood cell count for the diagnosis of culture-proven neonatal sepsis. *Scand J Infect Dis* 2002;34: 620-22.

93) Boetow KC, Klien SW, Lane RB, Septicemia in premature infants. Am J Dis Child, 196; 110 :29.

94) Gendrel D, Assicot M, Raymond J. Procalcitonin as a marker for the early diagnosis of neonatal infections. *J Paediatr* 1996;128:570-73.

95) Gluck L, Wood HF, Fousek MD., Septicemia of the Newborn Pediatr Clin North Am, 1966; 13 : 1131.

96) Hatherill M, Tibby SM, Sykes K, Turner C, Murdoch IA. The diagnostic markers of infection and the comparison of procalcitonin with C-reactive protein and the leucocyte count. *Arch Dis Child* 1999;81: 417-21.

97) Jaswal RS, Kaushal RK,Goel A, Pathania K. Role of the C-reactive protein in deciding the duration of the antibiotic therapy in neonatal septicaemia. *Indian Paediatrics* 2003;40:800-83.

98) Koksal N, Harmanci R, Getinkaya M. The roles of procalcitonin and CRP in the diagnosis and the follow up of neonatal sepsis cases : Turk J Paediatr 2007;49:21-9.

99) Malik A , Hassani SE , Khan H M , Ahmed A J , Nosocomial infection in new born. Ind. Paediatrics. 2001; 38:68-71.

100) Monneret G, Labaune JM, Isaac C. Procalcitonin and C-reactive protein levels in neonatal infections. *Acta Paediatr* 1997;86:209-12.

101) Rodwell RL, Leslie AL, Tudehope DL. Early diagnosis of neonatal sepsis by using a hematological scoring system. *J Paediatr* 1988;112:161- 66.

102) Sakha K, Husseini M.B, Seyyedsadri N. The role of procalcitonin in the diagnosis of neonatal sepsis and the correlation between procalciton in and C-reactive protein in these patients. *Pak J Biol Sci* 2008;11:1785-90.

103) Sanghvi KP,Tudehope DI. Neonatal bacterial sepsis in a neonatal intensive care unit: A 5 year analysis. J. Pediatr child health, 1996:32:333-338.

104) Sharma A. Evaluation of sepsis screen for diagnosis of neonatal septicemia. Indian J Paediatr 1993 Jul - Aug 60:559-63.

105) Stevens and colleagues. Early onset Pseudomonas sepsis of the neonate. Perinatal , neonatal 1982; 6:75.

106) Tallur SS,Kasturi AV , Nadgir , Krishna BV, Clinico bacteriological study of neonatal sepsis in Hubli. Indian J. Pediatr 2000;67:169-174.

107) Ugarte H, Silva E, Mercan D, DeMendonca A, Vincent J l: Procalcitonin used as a marker of infection in the intensive care unit. Crit care Med 1998;24: 1052-1056.

108) Vazzalwar R, Pina-Rodrigues E, Puppala BL, Angst DB, Schweig L. Procalcitonin as a screening test for late-onset sepsis in preterm, very low birth weight infants. *J Perinatol* 2005;25:397-402.

SNO	NAME	WEIGHT	DAYS	GENDER	REFUSAL OF FEEDS	FEVER	LETHARGY	INCESSANT CRY	SEIZURES	RD	VOMITTING	LOOSE STOOLS	ABDOMINAL DISTENSION	RISK FACTOR	DISEASE	EOS/LOS	XRAY CHANGES	PRETERM	NORMAL DELIVERY
1	B/O DEEPA		10	M	R	F	L							BAD CRP		LOS	Y	TERM	LSCS
2	REKHA		4	F	R			I		RD						LOS	Y	TERM	Y
3	GOMATHY	2.3	60HRS	F	R	F								PROM		EOS	NO	TERM	LSCS
4	INBANITHA		22	F	R	F	L									LOS	NO	TERM	Y
5	LAKSHMI LAVANYA	2.2	60HRS	F	R		L	I		RD	V			PROM		EOS	NO	PT	LSCS
6	VANITHA		20	M	R	F		I						PROM		LOS	NO	TERM	LSCS
7	GOWRI		10	M	R	F								BAD CRP		LOS	NO	TERM	Y
8	SASIREKHA		13	M	R	F	L							PROM		LOS	NO	TERM	Y
9	JAYAKODI		28	F	R		L		S						MENINGITIS	LOS	NO	PT	Y
10	SUNITHA	2.2	1	F	R		L			RD				PROM /PIH		EOS	NO	PT	LSCS
11	BUVANESWARI MAGESH	1.9	1	F	R			I		RD				VLBW	BRONCHO PNEUMONIA	EOS	Y	PT	Y
12	LINGESH		21	M	R	F					V		D	MATERNAL FEVER	SEPTIC ILEUS	LOS	Y	PT	LSCS
13	RADHIKA		6	F	R											LOS	NO	PT	Y
14	SANTHOSH		19	M		F		I		RD				MECHANICAL VENTILATION		LOS	Y	PT	LSCS
15	VENNILA	1.8	1	F				I		RD				HOME DELIVERY		EOS	NO	PT	Y
16	BAVANI		25	F	R	F		I		RD					BRONCHO PNEUMONIA	LOS	NO	PT	LSCS
17	NANDINI		16	M	R			I		RD					BRONCHO PNEUMONIA	LOS	NO	PT	Y
18	SARASWATHI		20	F		F		I		RD			D			LOS	NO	TERM	LSCS
19	ESWARI		11	M	R											LOS	NO	TERM	Y
20	JAYAPRIYA		21	F	R	F			S					PROM	MENINGITIS	LOS	NO	TERM	LSCS
21	LALITHA MAGESWARI		7	M	R	F									BRONCHO PNEUMONIA	LOS	Y	TERM	LSCS
22	AYYANAR		17	M	R	F	L						D	BAD CRP	MENINGITIS	LOS	NO	TERM	LSCS
23	ESTHER RANI	2.3	1	F	R	F				RD	V			VLBW		EOS	NO	TERM	LSCS
24	JAYA MARY		15	F							V		D		NNEC	LOS	Y	PT	LSCS
2	KATHIR		20	M	R	F			S		V				PYELONEPHRITIS	LOS	NO	TERM	LSCS
26	RANI		4	F	R	F	L									LOS	NO	TERM	LSCS
27	KALAIVANI		5	M	R	F							D			LOS	NO	TERM	LSCS
28	USHA		5	F	R	F	L								BRONCHO PNEUMONIA	LOS	NO	PT	LSCS
29	PARAMESWARI		28	F	R											LOS	NO	PT	Y
30	SURYA		28	M	R			I		RD					BRONCHO PNEUMONIA	LOS	Y	TERM	Y
31	SAVITHRI		15	F		F	L		S					MATERNAL FEVER	MENINGITIS	LOS	NO	PT	LSCS
32	SANGEETHA		21	F		F				RD						LOS	NO	TERM	LSCS
33	MARUSELVI		12	M	R	F										LOS	NO	TERM	LSCS
34	KAVITHA	2	2	M		F				RD			D	HOME DELIVERY		EOS	NO	PT	Y
35	JAYANTHI		6	F	R	F								PROM		LOS	NO	PT	LSCS
36	PARAMESWARI		20	F	R					RD						LOS	NO	TERM	Y
37	JAYANTHI	1.9	2	M	R	F				RD		LS		VLBW		EOS	NO	TERM	Y
38	TEJAVATHI		5	M	R	F							D	MECHANICAL VENTILATION		LOS	Y	PT	LSCS
39	MOHAMAD SAMEER		5	M	R					RD						LOS	NO	TERM	LSCS
40	MADHURI LATHA		22	F	R					RD						LOS	Y	TERM	LSCS
41	GOWTHAM		28	M		F				RD				MATERNAL FEVER		LOS	NO	TERM	Y
42	RANI		23	M	R	F	L									LOS	NO	PT	LSCS
43	GUNASRI		25	F	R					RD	V				BRONCHO PNEUMONIA	LOS	NO	TERM	Y
44	VIGNESWARAN		23	M	R					RD						LOS	NO	PT	Y
45	SELVI		20	F	R					RD						LOS	NO	TERM	LSCS
46	LAKSHMI PRASANNA	2.6	1	M	R					RD				PROM		EOS	NO	TERM	LSCS
47	KANAGA		28	M	R	F							D	MATERNAL FEVER		LOS	NO	TERM	Y
48	KOUSALYA		17	F		F				RD						LOS	NO	TERM	Y
49	VIGNESH		24	M	R					RD					BRONCHO PNEUMONIA	LOS	Y	TERM	Y
50	DOWLINA		28	M	R							LS				LOS	NO	TERM	Y
51	VISWAJITH		27.000	M	R					RD					BRONCHO PNEUMONIA	LOS	NO	TERM	Y

52	SANGEETHA		5	M	R					RD						LOS	NO	TERM	Y
53	RIYALAKSHMI		27	M		F	L		S							LOS	NO	TERM	LSCS
54	DIWAKAR		22	M		F	L			RD						LOS	Y	TERM	Y
55	PRIYA		22	F	R					RD						LOS	Y	PT	Y
56	JAYAPRIYA		12	F	R					RD						LOS	NO	TERM	Y
57	NAVANEEDHAM		9	M	R											LOS	NO	TERM	LSCS
58	HEMANJINI		20	F		F				RD				PROM	BRONCHO PNEUMONIA	LOS	Y	PT	Y
59	DEEPA		24	F	R	F			S					MATERNAL FEVER	MENINGITIS	LOS	NO	TERM	LSCS
60	SUREKHA	2.24	1	F	R					RD				PROM		EOS	NO	TERM	Y
61	NIVEDHA		28	M	R	F									SEPTIC ARTHRITIS	LOS	NO	TERM	Y
62	MUNUSAMY		21	M	R	F				RD					BRONCHO PNEUMONIA	LOS	Y	TERM	Y
63	KRISHNAVANI		28	F	R	F			S						MENINGITIS	LOS	NO	TERM	Y
64	CHITHRA		26	F	R	F									SEPTIC ARTHRITIS	LOS	NO	TERM	LSCS
65	JEEVA		26	M	R	F	L			RD					MENINGITIS	LOS	NO	PT	Y
66	ALLABAKESH	2.3	1	M	R					RD				VLBW		EOS	NO	PT	Y
67	MEGALA		5	M		F				RD						LOS	NO	TERM	Y
68	MALLIGA		28	M		F			S	RD						LOS	NO	PT	Y
69	ANITHA		20	F		F				RD			D		NNEC	LOS	NO	TERM	LSCS
70	POORNIMA		15	F		F							D			LOS	Y	TERM	Y
71	SUMITHRA		10	M	R					RD						LOS	NO	TERM	Y
72	RANJITHA		7	F		F						LS	D			LOS	NO	TERM	Y
73	GEETHA		28	F	R					RD						LOS	Y	PT	Y
74	UMA		23	M	R	F										LOS	Y	TERM	Y
75	RAMA		20	M	R	F										LOS	Y	TERM	LSCS
76	KALAIVANI		1	F	R					RD						LOS	NO	PT	Y
77	PUNITHA		2	M	R											EOS	NO	PT	Y
78	NAGAMMAL		4	F	R	F										LOS	Y	PT	Y
79	DIVYA		10	F	R	F										LOS	NO	TERM	LSCS
80	KAVITHA		4	F	R	F										LOS	Y	PT	Y
81	ANITHA		12	F	R					RD						LOS	Y	PT	Y
82	B/O SELVI		14	F	R					RD						LOS	NO	PT	Y
83	NANDHINI		20	F	R	F				RD	V					LOS	Y	PT	Y
84	BAWANI I		10	M						RD						LOS	Y	TERM	Y
85	BHARATHI		28	F	R					RD						LOS	NO	TERM	LSCS
86	PREMALATHA		12	M	R					RD					BRONCHOPNEUMONIA	LOS	Y	TERM	Y
87	INDUMATHY		8	F	R					RD						LOS	NO	PT	LSCS
88	THILAGAM		6	F	R											LOS	NO	TERM	LSCS
89	SAROJINI		10	F						RD						LOS	NO	PT	Y
90	KALAIVANI		1	F	R					RD					BRONCHOPNEUMONIA	EOS	Y	PT	Y
91	SHAMEEMA		10	M	R					RD						LOS	NO	PT	Y
92	BHUVANESWARI		2	F						RD						EOS	NO	PT	Y
93	HEMA		12	M	R										NNH	LOS	NO	PT	Y
94	KALAIVANI		5	F												LOS	NO	PT	Y
95	KALAISELVI		2	F						RD						EOS	NO	PT	LSCS
96	PARVEEN		13	F						RD						LOS	NO	PT	Y
97	SINDHAMANI		10	F	R					RD					NNH	LOS	NO	TERM	LSCS
98	DIVYA		6	F	R					RD						LOS	NO	TERM	Y
99	KALAIVANI		4	M	R					RD					NNH	LOS	NO	TERM	Y
100	POORNIMA DEVI		28	F		F				RD					BRONCHOPNEUMONIA	LOS	NO	TERM	Y

NORMAL WEIGHT/ LBW	ALTERED HAEMOTOLOGICAL PARAMETERS	CRP QUANTITATIVE	CRP QUALITATIVE	PCT < 0.25 NEGATIVE	PCT < 0.5	PCT 0.5 - 10	PCT >10	ORGANISM	AK	GM	PEN	ERY	COTRI	CTX	CEFTAZIDIME	CIP	CEFOXITIN	IMI	PT	VAN	CHLORAMPHENICOL		AMPI	HLG
N	Y	5.964	+				10.5	NG																
LBW	Y	1.621	+			1.14		NG																
LBW	NO	4.32	+			0.99		NG																
N	Y	5.6	+			0.65		NG																
LBW	NO	9.8	+			2.94		GNB -KLEBSIELLA PNEUMONIAE(ESBL)	S	R	_	_	R	R	S	S	_	S	S	_	_	_		
N	NO	1.62	-	NEG				NG																
N	NO	1.91	-	NEG				NG																
N	Y	24.4	+				54	GNB PESUDOMONAS STUTZERI	S	R	_	_	_	R	S	S	_	S	S	_	_	_		
LBW	NO	5.86	-			5.3		NG																
LBW	NO	18.4	-			8.93		NG																
LBW	NO	4.23	-		0.3			NG																
N	NO	1.81	-	NEG				NG																
LBW	NO	0.28	-	NEG				NG																
N	NO	1.42	-			0.56		GNB- KLEBSIELLA PNEUMONIAE	S	R	_	_	R	S	S	S	_	S	S	_	_	_		
LBW	NO	3.9	+	NEG				NG																
LBW	NO	5.43	-			6.35		NG																
N	NO	3.23	+	NEG				NG																
LBW	NO	4.72	-			0.85		NG																
N	NO	5.43	-			0.6		NG																
LBW	Y	20.3	+			4.03		GPC - STAPH AUREUS (MSSA)	S	R	S	R	R	_	_	S	S	_	_	S	_	_		
LBW	Y	5.9	-			7.3		PSEUDOMONAS AERUGINOSA	S	S	_	_	_	R	S	S	_	S	S	_	_	_		
N	Y	32	+			6.3		NG																
LBW	NO	5.12	-			1.12		NG																
LBW	NO	4.72	-	NEG				GNB-KLEBSIELLA PNEUMONIAE(ESBL)	S	R	_	_	R	R	S	S	_	S	S	_	_	_		
LBW	Y	8.9	+			2.96		GNB ACINETOBACTER BAUMANI	S	R	_	_	R	R	S	R	_	S	S	_	_	_		
LBW	NO	14.86	-	NEG				NG																
LBW	NO	1.82	+				28.1	GNB- KLEBSIELLA PNEUMONIAE	S	R	_	_	R	S	S	S	_	S	S	_	_	_		
LBW	NO	25.3	+	NEG				NG	R	R	_	_	R	_	_	_	_	_	_	_	_	_		
LBW	Y	8.245	-	NEG				GNB ESCHERICHIA COLI	S	R	_	_	R	S	S	S	_	S	S	_	_	_		
LBW	NO	3.11	-	NEG				NG																
N	Y	1.89	-			3.27		GNB ACINETOBACTER BAUMANI	R	R	_	_	R	R	R	R	_	S	S	_	_	_		
LBW	Y	11.4	+			5.11		GNB- KLEBSIELLA PNEUMONIAE	R	R	_	_	R	S	S	S	_	S	S	_	_	_		
LBW	NO	10.2	-		0.26			NG																
LBW	Y	2.9	+			3.01		NG																
N	Y	53.8	+			0.8		NG																
N	Y	9.82	+			2.01		NG																
LBW	NO	17.2	+			1.7		NG																
N	Y	11.32	-		0.43			NG																
LBW	NO	4.9	+				13	NG																
N	NO	21.8	+			1.8		NG																
N	NO	11.18	-	NEG				NG																
LBW	Y	2.32	+			1.26		NG																
N	NO	8.22	-	NEG				NG																
LBW	NO	3.92	+			1.72		GPC - ENTEROCOCCUS FAECALIS	_	_	R	R	_	_	_	_	_	_	_	S	_	_	R	S
LBW	NO	11.7	+				19	GPC - STAPH AUREUS(MRSA)	S	R	R	R	R	_	_	S	R	_	_	S	_	_		
N	Y	23.89	-	NEG				NG																
N	Y	1.92	-	NEG				NG																
LBW	Y	2.58	-	NEG				NG																
N	NO	3.024	-			0.67		NG																
LBW	NO	5.89	+			1.92		NG																
N	NO	4.724	-	NEG				NG																

LBW	NO	4.89	-	NEG				NG																	
N	NO	3.94	+	NEG				NG																	
N	Y	9.2	-			2.63		NG																	
LBW	Y	4.928	+			3.01		NG																	
LBW	NO	15.24	-			0.65		NG																	
LBW	Y	5.432	+			5		GNB-KLEBSIELLA PNEUMONIAE(ESBL)	S	R	_	_	_	R	S	R	_	S	S	_	_	_		
LBW	NO	29.524	+			9.99		NG																	
N	Y	22.4	+			9.9		GNB ESCHERICHIA COLI	S	R	_	_	R	R	R	R	_	S	S	_	_	_		
LBW	NO	30.2	+			4.32		GNB ACINETOBACTER BAUMANI	S	R	_	_	R	S	S	S	_	S	S	_	_	_		
N	Y	29.12	+			1.62		GPC- STAPH AUREUS(MSSA)	S	R	R	R	R	_	_	S	S	_	_	S	_	_		
N	NO	11.245	+				11.3	GPC- STAPH AUREUS(MRSA)	S	R	R	R	R	_	_	S	R	_	_	S	_	_		
LBW	NO	18.41	+			8.32		NG																	
N	Y	18.12	+			0.58		NG																	
LBW	Y	9.02	-			0.9		NG																	
LBW	Y	8.34	-			0.6		NG																	
N	NO	4.824	+			0.85		NG																	
LBW	NO	7.28	-	NEG				NG																	
N	Y	4.32	+			3.02		GPC- STAPH AUREUS(MSSA)	S	R	R	R	R	_	_	S	S	_	_	S	_	_		
N	NO	12.43	-	NEG				GNB ACINETOBACTER BAUMANI	R	R	_	_	R	R	S	R	_	S	S	_	_	_		
N	NO	3.3	-	NEG				NG																	
N	NO	4.92	-			YES		NG																	
LBW	NO	4.47	_	NEG				NG																	
N	NO	9.2	+		YES			GPC-CONS	S	R	S	R	R	_	_	S	_	_	_	_	_	_	_	_	_
N	NO	7.3	-			YES		NG																	
LBW	NO	8.34	+	NEG				NG																	
LBW	NO	6.98	+			YES		NG																	
LBW	NO	5.8	-			YES		NG																	
N	NO	8.24	+			YES		NG																	
LBW	NO	8.9	-			YES 1.8		GPC-CONS	S	R	S	R	R	_	_	S	_	_	_	_	_	_	_	_	_
LBW	Y	20.01	+				YES	NG																	
LBW	NO	5.8	-			YES		PSEUDOMONAS AERUGINOSA	S	S	_	_	_	R	S	S	_	S	S	_	_	_		
LBW	NO	14.01	-			YES		PSEUDOMONAS AERUGINOSA	S	S	_	_	_	R	S	S	_	S	S	_	_	_		
LBW	NO	10.94	+			YES		NG																	
LBW	NO	9.124	-			YES		NG																	
LBW	NO	3.2	_	NEG				NG																	
LBW	NO	4.92	_	NEG				NG																	
N	NO	2.23	-	NEG				NG																	
LBW	NO	5.9	-	NEG				NG																	
LBW	NO	2.8	+	NEG				NG																	
LBW	NO	4.74	-	NEG				NG																	
LBW	Y	3.82	-	NEG				NG																	
LBW	NO	5.01	-	NEG				NG																	
LBW	NO	8.8	-			0.65		NG																	
LBW	NO	4.3	-	NEG				NG																	
LBW	NO	1.014	-	NEG				NG																	
LBW	NO	2.79	-	NEG				NG																	
LBW	NO	3.82	-	NEG				NG																	
LBW	NO	4.39	-	NEG				NG																	
N	Y	10.4	-			0.85		NG																	